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Aerial dispersion of *Xanthomonas fragariae* during trimming leaves of angular leaf spot diseased strawberry propagation plants

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Summary The bacterium *Xanthomonas fragariae* is the causative agent of angular leaf spot (ALS) of strawberry, a Regulated Non-Quarantine Pest in Europe (EPPO A2) for plant propagation material. Field experiments were conducted to explore if *X. fragariae* is dispersed through the air during trimming ALS-diseased strawberry plants with dry or wetted leaves. Trimming the leaves led to dissemination of leaf fragments to the nearby surroundings. A sharp decrease in the amount of leaf fragments within the first 5 m distance downwind from the strawberry plants was found. Furthermore, air quality monitors demonstrated that during trimming 0.5 – 10 µm sized particles were ejected into the air, resulting in short periods with increased particle densities 45 cm above ground level, which could be detected at least 50 m downwind. At this height *X. fragariae* was detected by means of air samplers, as evidenced with a combination of dilution-plating and TaqMan assays, at 25 m distance downwind from ALS-diseased plants. A sharp decrease in the density of *X. fragariae* colony forming units (cfu) within the first 10 m distance from the source plants was found. The densities of *X. fragariae* cfu were strongly associated with the particle densities. Results indicate that during trimming leaves of strawberry propagation crops with ALS-diseased plants there is a considerable risk of deposition of airborne *X. fragariae* inoculum on nearby nursery beds. Whether this airborne inoculum can result in infections is discussed.

Keywords *Fragaria* x *ananassa* · Mechanical leaf trimming · Trimming clouds · Air sampling · Quantification of emitted aerosol particles · TaqMan tests

Introduction

Xanthomonas fragariae is the causative agent of angular leaf spot disease of strawberry (Kennedy & King, 1962a). Although the bacterial pathogen is found in many countries in Europe, for propagation material imported from countries outside the EPPO region *X. fragariae* is still considered as a quarantine pathogen and therefore put on the EPPO A2 list (Suffert, 2012). However, in 2018 within the European Union the pathogen was down-regulated into a Regulated Non-Quarantine Pest (RNQP), for the presence of which visual inspection and, where applicable, supplementary sampling and testing with appropriate diagnostic methods is required (Picard et al., 2018). The use of latently infected propagation material is considered

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the primary method by which *X. fragariae* is introduced in new strawberry plantings (Pooler et al., 1996; Roberts et al., 1998; Wang & Turechek, 2016). When the pathogen becomes active, secondary spread in the field may occur via splashing water during rain and overhead irrigation or via contact with contaminated machinery, clothes and skins of animals (Maas, 2004). As a result of the implementation of a rigid certification scheme (EPPO, 2008) and inspection procedure for plant-propagation nurseries (EPPO, 2017), numbers of *X. fragariae* infections found in strawberry propagation material in the Netherlands decreased from 73 cases in 2012 (Van der Sande, 2016) to 7 in 2017 (Van der Sande, 2018).

In preparation for the harvest of certified waiting bed plants, the leaves of the strawberry plants are trimmed and the daughter plants are cut loose from the mother plants. Kastelein et al. (2018) demonstrated that *X. fragariae* can be spread over a propagation bed by a rotary mower that comes into contact with angular leaf spot (ALS) affected plants. In that study indications were also found for wind-dispersion of the pathogen to neighbouring beds with strawberry plants. However, experimental evidence for the supposed aerial dispersion of *X. fragariae* is lacking in the published literature. Experimental proof will support the development of more effective control strategies to restrict further spread of the pathogen during the production of strawberry propagation material.

The aim of this research was to confirm the aerial dispersion of *X. fragariae* during trimming ALS-affected leaves of strawberry plants grown as a propagation crop. Experiments were conducted under field conditions with plants with either dry or wetted leaves. During the time shortly before trimming, in the course of trimming and shortly after trimming ALS-affected plants, the densities of 0.5 – 10 µm sized particles and the presence of *X. fragariae* in the air were determined at various distances downwind from these plants. After the trimming runs, the presence of *X. fragariae* in leaf fragments which settled on the ground was assessed.

Materials and methods

Xanthomonas fragariae and culturing

The natural Rifampicin resistant strain 3488 of *X. fragariae*, already used in previous field experiments (Kastelein et al., 2018; Van der Wolf et al., 2018), was also used in present field experiments. Strain 3488 was stored and revived as described previously.

Inoculum was prepared by growing strain 3488 on R2AGR [18.12 g L^{-1} R2A Agar (Difco, USA), 25 mg L^{-1} glycine (Sigma-Aldrich, USA) and 50 mg L^{-1} Rifampicin (Duchefa Biochemie, NL)] for 3 days at 25 °C, washing the cells from the agar with 2 times 2 mL quarter-strength Ringer solution (Oxoid, UK) per plate and diluting the milky cell suspension in tap water to $10^9 - 10^{10}$ cfu mL^{-1}. Determination of the dilution factor was based on the experience (Kastelein et al., 2014) that cell suspensions diluted to an absorbance value of $A_{600nm} = 0.1$ had a density of approximately 10^8 cfu mL^{-1}.

Cells of *X. fragariae* in an air sample or leaf extract were enumerated by spread plating a dilution series on R2AGRC [R2AGR supplemented with 200 mg L^{-1} cycloheximide (Duchefa Biochemie)]. Plates were incubated for 8 – 10 days at 25 °C before scoring numbers of *Xanthomonas*-like colonies (circular, convex, glistening and translucent to pale-yellow).

Strawberry plants and inoculation

On 16 September 2015 young Elsanta tray plants were obtained from a commercial strawberry propagator. A total of 500 trays (100 × 19.5 × 11 cm), each consisting of 16 cups with one well developed strawberry plant, was transferred to a paved open-air nursery of Wageningen University and Research for continued growth.

Spread over mid-September and mid-October a total of four batches of 80 trays were transferred to a greenhouse compartment with temperatures fluctuating around 20 °C and a relative humidity of 85%. The trays were placed in humid chambers made of a metallic frame covered with transparent plastic sheeting. Relative humidity in the chambers ranged from 90% at daytime to saturated during the night. Inoculations were done 2 days thereafter. Bacterial inoculum to which 0.3% Silwet L-77 (GE Silicone, USA) surfactant was added to improve spreading out of the liquid, was atomized with a 1.25 L air pressure houseplant sprayer (Gardena, DE) on both sides of the leaves to run off. Three days after inoculation the humid chambers were opened to allow the leaves to dry. One week after inoculation the plants were transferred to a screenhouse, in which they were kept until

being used as source of inoculum of *X. fragariae* in field experiments with pathogen dispersal during leaf trimming. Within three weeks after inoculation most tray plants developed symptoms of ALS.

Leaf trimming experiments

On four different days between the end of October and mid November 2015 four leaf trimming experiments, in which the trimming of the foliage of young strawberry plants on propagation beds in preparation to their harvest was imitated, were carried out on a well-cut grass plot at Nergena experimental farm near Wageningen. The trial site is located in an area of the Netherlands where no strawberries are grown on a commercial scale. Experimental work was done during dry weather conditions with light (1 – 3 m s^{-1}) to moderate (4 – 8 m s^{-1}) breezes. During the 1st experiment the wind was blowing from a southern direction and conditions changed from sunny to cloudy. The other experiments were carried out on cloudy days. During the 2nd experiment the wind was blowing from a south-eastern direction. On the 3rd and 4th day a more westerly wind was blowing. During the time the experimental work was done no numerical data about the meteorological conditions were recorded at the grass plot of Nergena. However, Table S1 describes the meteorological conditions at a weather station at 3 km beeline distance from the experimental plot during experimental work in the field. In addition, Table S1 describes the background densities of microscopic particles in the air above the experimental plot during the periods between sessions in which the leaves of strawberry plants were trimmed.

Each experiment consisted of four sessions in which the leaves of strawberry plants were trimmed using a tractor-operated rear-mounted Perfect LF215 rotary mower with cutter blades (Van Wamel b.v., NL). The mower, which was adjusted to clip off approximately the upper two thirds of the foliage, had facilities that prevented sideward spread of the clipped plant parts. Most clippings came down between the two closed sides of the mower on the trays with strawberry plants. However, some haulm fragments (i.e. leaf and petiole fragments) were ejected into the air through the 30 cm high and 200 cm broad open back of the mower. After each trimming run air samples and windblown haulm fragments which settled down on the ground were sampled at the various distances downwind from the trays with strawberry plants mentioned below in the section 'Sampling wind-dispersed haulm fragments and sample processing'.

In each trimming run 24 trays (four groups of six trays, each with two trays after each other and three side by side) arranged along a line across the preponderant wind-direction of the day (leaving a gap of 30 cmbetween each six-tray group; Fig. 1) were trimmed. In the 2nd and 4th experiment the leaves of the strawberry plants were wetted shortly before each trimming run using a garden spray gun to imitate trimming after a shower of rain. When the wind was turning in different directions trimming runs were postponed until the wind was blowing again from the preponderant wind-direction. After each run the rotary mower was cleaned with water by hosing the debris from the cutter blades. On each experimental day a group of non-inoculated control plants were trimmed first. The other three trimming runs (2 Xf1, 3 Xf2 and 4 Xf3) on an experimental day were carried out with ALS-affected plants. After mowing, the trays with the trimmed plants were replaced by a new set of plants. The strawberry plants only stayed in the field during the time needed to do the experimental work of that day.

Assessment of the source strength

In each experiment the density of *X. fragariae* in the leaves of strawberry plants was assessed for a random selection of inoculated plants. For this, shortly after putting the trays for a trimming run with *X. fragariae* inoculated plants at their place, of each group of six trays one plant was removed and individually packed in a plastic bag and processed later that experimental day.

The blades of the leaves were clipped off and transferred to an universal extraction bag (Bioreba, CH). After assessing the sample weight and crushing the leaf tissues with a hammer, a volume of Wilbrink's solution (Kastelein et al., 2018) equivalent to 1.3 times the sample weight was added and mixed through the leaf tissues. The leaf extract was incubated 10 – 15 min before plating 50 µL of 100-fold serial dilutions (up to one million times diluted) in quarter-strength Ringer suspension on R2AGRC. After incubation as described above in '*Xanthomonas fragariae* and culturing' *Xanthomonas*-like colonies

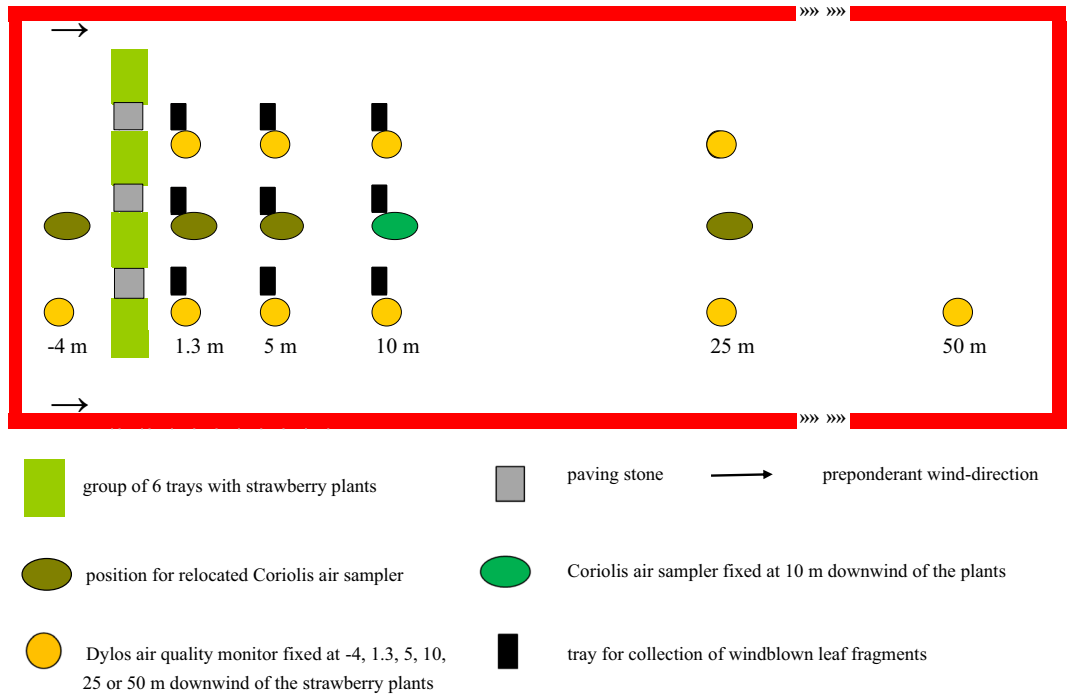

Fig. 1 Diagram of an experimental plot in which the aerial dispersion of *Xanthomonas fragariae* during trimming leaves of angular leaf spot diseased strawberry propagation plants was studied. The orientation of the plot is shown relative to the preponderant wind-direction during the experiment

were counted and the density of *X. fragariae* cfu g^{-1} leaf tissue was calculated. The average of the *X. fragariae* densities in the leaf tissues of the 12 plants per experiment was used to estimate the source strength.

Sampling wind-dispersed haulm fragments and sample processing

To enable sampling haulm fragments and other crushed plant material which settle down on the ground between the strawberry plants during trimming them, before each trimming run paving stones (28.5 × 28.5 cm) were placed between the groups of trays with strawberry plants. For the purpose of sampling wind-dispersed haulm fragments which settled down on the ground, before each trimming run plastic dinner-trays (44.5 × 29 × 1 cm; l x w x h) were placed downwind on the grass plot along three lines laid down from the three paving stones within the row of strawberry plants (Fig. 1). The lines were laid down with in-between distances of 2.3 m parallel to the preponderant wind-direction during the experiment. Dinner-trays were placed at 1.3 m (trimming non-inoculated control plant as well as ALS-affected plants), 5 and 10 m (ALS-affected plants, only) away from the strawberry plants.

Within 5 – 10 min after each trimming run, the material detectable with the unaided eye on the paving stones (i.e. haulm fragments and crushed plant material not blown away by the wind) and in the dinner-trays (i.e. haulm fragments transported by the wind) was collected in pre-weighed 180 mL plastic containers (Greiner BioOne, AT), during which the material of the three samples per distance from the trimmed strawberry plants was pooled into one composite sample. The samples collected from the paving stones between the plants were processed per trimming run. Because of the small amounts of material collected in the trays at 1.3, 5 and 10 m distance, all the material collected during the three trimming runs with ALS-affected strawberry plants was pooled per distance from the trimmed plants before further processing.

Weighed plant parts were agitated in 100 mL of quarter-strength Ringer solution on a Stuart SF1 Flask Shaker (Bibby Scientific Ltd, UK) for 10 min at

800 oscillations min^{-1}. If a sample exceeded 20 g, a subsample was processed. Bacterial cells released in the washing medium were concentrated 50× by centrifugation. To do this the washing medium was transferred to a sterile 250-mL centrifuge bottle, spun at 12296 RCF for 15 min at 10 °C in a SLA-1500 rotor of a Sorvall RC 6 Plus centrifuge (ThermoFisher Scientific), after which the supernatant was drained and the pellet suspended in 2 mL of quarter-strength Ringer solution. To check for the presence of *X. fragariae* in the plant extract, a Direct TaqMan test and a plating test were used. For the Direct TaqMan test, 1 ml undiluted extract was transferred into a 1.2 mL tube of a 12×8 microtube storage rack (Qiagen Benelux, NL) and processed as described below in the section 'Direct, Bio- and Colony-Taqman tests'. For the plating test 50 µL undiluted extract and a 100, 10,000 and 1,000,000 fold dilution in quarter-strength Ringer solution was plated on R2AGRC. For the non-inoculated controls only undiluted and 100× diluted plant extract was plated. After incubation the identity of a randomly selected *Xanthomonas*-like colony was checked by a Colony-TaqMan test. Based on the results of the Colony-TaqMan test and the colony count the density of *X. fragariae* cfu m^{-2} ground surface was calculated. When no *Xanthomonas*-like colonies were observed on plates seeded with the dilution series of a haulm extract the absence of *X. fragariae* in the sample was confirmed by a Bio-TaqMan test.

Sampling air for the presence of *X. fragariae* and sample processing

Two Coriolis Micro air samplers (Bertin Instruments, FR) were placed on platforms arranged along the line laid down from the middle of the row of strawberry plants in the main direction of the wind (Fig. 1). The air intake of the samplers was situated at a distance ranging from 4 m upwind (before trimming control plants, only) and 1.3 m to a maximum of 25 m downwind from the strawberry plants at a height of 47 cm above ground level and turned into the wind. One sampler was placed at 10 m downwind from the strawberry plants during the whole duration of each experiment. The other sampler was relocated at 25 m (runs 1 and 2, with control and ALS-affected plants, respectively), 5 m (run 3 with ALS-affected plants) and 1.3 m (run 4 with ALS-affected plants) downwind between the trimming runs. The capacity of the Coriolis samplers was set at sampling 300 L air per minute. During a period of 3 min, starting at the beginning of each leaf trimming run, the particles present in total of 0.9 m^3 air were collected in a cone (Bertin Instruments) filled with 15 mL RT [quarter-strength Ringer solution with 0.01% Tween20 (ThermoFischer Scientific, USA)].

The density of the particles in the air samples was concentrated tenfold by centrifugation. To do this the samples were transferred to sterile 50 mL Nunc conical centrifuge tubes (ThermoFisher Scientific), spun at 8867 RCF for 10 min at 10 °C in a Fiberlite F-15–6×100y rotor of a SL40R benchtop centrifuge (ThermoFisher Scientific), after which the supernatant was drained and the pellet suspended in 1.5 mL quarter-strength Ringer solution.

The presence of *X. fragariae* in the concentrated air samples was determined by means of a Direct TaqMan test and a plating test as outlined above for wind-dispersed haulm fragments. Based on the colony counts and the average of the duration of the trimming cloud passing over the two Dylos DC1700 air quality monitors (Dylos Corporation, USA) at both sides of the Coriolis air samplers the densities of *X. fragariae* cfu m^{-3} air were calculated.

Monitoring aerosol particles and data processing

Dylos DC1700 air quality monitors were used to quantify aerosol particles in the trimming cloud, i.e. the cloud of microscopic particles ejected into the air during trimming strawberry plants. Air quality monitors were placed at 1.3, 5, 10 and 25 m distance downwind from the row of the strawberry plants on platforms arranged along the two lines laid down from the paving stones nearest to the ends of the row of strawberry plants (Fig. 1). Furthermore, one air quality monitor was placed at 4 m upwind and one monitor at 50 m downwind of the strawberry plants. The air intake of the monitors was situated at a height of 45 cm above ground level and the aperture turned into the wind.

The Dylos air quality monitors assessed numbers of particles of size distributions 0.5 – 10 µm and 2.5 – 10 µm per cubic foot (cf). Recordings were the average of 10 s measurements. Each minute 6 recordings of the densities of these microscopic particles in

the air, date and time were stored in the data base of the monitors. During the experiments all 10 air quality monitors made continuous readings from about 10 min before the start of the first trimming run onwards till about 10 min after the end of the last trimming run. Thus, data of particle densities in the air were recorded when no strawberry plants were trimmed.

For each recording, data processing started with the calculation of the density of particles of size distribution 0.5 – 2.5 µm (below referred to as fine particles) by subtracting the reading for particles of size distribution 2.5 – 10 µm (below referred to as coarse particles) from the reading for particles of size distribution 0.5 – 10 µm and by representing the densities of fine and coarse particles in XY scatter plots with time and density of particles along the X- and Y-axis, respectively. For the definitions of fine and coarse particles we followed those suggested by Whitby (1978). Although the densities of fine and coarse particles fluctuated continuously, every cloud of microscopic particles ejected into the air during the four trimming runs of an experiment was recognizable by distinct peaks in the densities of both categories of particles in the scatter plots of data from the air quality monitors placed at distances 1.3 and 5 mdownwind from the strawberry plants. For the monitors placed at 10 mdownwind this was the case for most trimming runs. In the plots of the data of the monitors placed at 25 and 50 mdownwind, at most two out of the four clouds of microscopic particles (below referred to as trimming clouds) were recognizable by a distinct peak. For the cases in which a trimming run was not recognizable as distinct peak in densities of particles, the data of the 4 – 5 monitors along one line were aligned with the monitors at the corresponding distance along the other line to determine whether the passage of the trimming clouds involved could be distinguished as an reduced increase in densities of the particles. For each air quality monitor and trimming run, the densities of fine and coarse particles in a trimming cloud were estimated in a two stage procedure. The background density (density when no plants were trimmed) of each category of particles was determined by calculating the average of the six readings directly before the onset of the peak and the six readings directly after the end of the drop of the peak in particles associated with the trimming run. The background densities of the particles were then subtracted from the 3 – 5 peak readings followed by adding up the results of these subtractions. To determine the interrelationship between the densities of fine and coarse particles (numbers cfu^{-1}) in the trimming clouds of ALS-diseased plants and the densities of *X. fragariae* (cfu m^{-3}) in the air the numbers per cubic foot of both categories of particles were converted to numbers per cubic metre. An insight of the interrelationships between the densities of fine as well as of coarse particles in the trimming clouds and the densities of *X. fragariae* in the air is of significance because the size category of fine particles includes aerosols of single bacterial cells.

Direct, bio- and colony-TaqMan tests

Direct TaqMan tests were done on extracts of wind-dispersed haulm fragments and concentrated samples of Coriolis air samplers. The Direct TaqMan test included transferring 1 ml of a liquid sample into one of the 1.2 mL tubes of a 12×8 microtube storage rack (Qiagen Benelux), sealing the microtubes, spinning the racks at RCF 4800×g for 15 min in a 152/02 Sigma-Qiagen rotor of a 4–15 C benchtop centrifuge (Sigma-Aldrich) and removal of 900 µL supernatant from each tube before storage of the pellets in the microtubes at − 20 °C until DNA extraction, DNA purification and performing a Taqman assay specific for *X. fragariae*.

Bio- and Colony-Taqman tests were done after the assessment of the results of the plating tests on extracts of windblown haulm fragments and concentrated samples of Coriolis air samplers. The Bio-TaqMan test included flooding of plates, seeded with undiluted or 100×diluted extract of haulm fragments or air sample, with 2.5 mL of quarter-strength Ringer solution, dislodging the bacterial colonies from the agar with the aid of an L-shaped spreader. Depending on the number of colonies 1 mL undiluted suspension, or diluted to a slightly clouded suspension, was transferred into a 1.2 mL tube of a 12×8 microtube storage rack. After sealing the microtubes, the racks with bacterial suspensions were processed as described above for the Direct TaqMan test. In the Colony-TaqMan test, bacterial cells from

Xanthomonas-like colonies were sampled with an inoculation needle, suspended in a 1.2 mL microtube containing 1 mL quarter-strength Ringer solution and processed as described above for the Direct TaqMan test.

DNA extraction and purification

After defrosting the stored pellets of plant extracts, concentrated air samples and suspensions of bacterial colonies the DNA was extracted using the Quick Pick XL Plant DNA extraction kit (Bio-Nobile, FI) as described in Kastelein et al. (2018).

To remove impurities, the DNA extract was filtered through a polyvinylpyrrolidone (PVPP) column. The wells of a multiscreen HTS 96-well HV filter plate with hydrophilic Durapore PVDF 0.45 µm pore size membranes (Merck Millipore, USA) were filled with PVPP powder (Sigma-Aldrich) through a mould (Wageningen Plant Research, home made). To moisten the PVPP, the multiscreen plate was placed on top of a 96-well ELISA microplate (Greiner BioOne) after which 150 µL of distilled water (Invitrogen, USA) was added per well and the multiscreen-ELISA plate combination centrifuged (152/02 Sigma-Qiagen rotor of a 4–15 C benchtop centrifuge) for 4 min at RCF 840×g, using the ELISA plate to collect free water. This was repeated once more. After moistening the PVPP the multiscreen plate was placed on top of a 96-well PCR microplate (Greiner BioOne) and 100 µL DNA extract was added to each well. The multiscreen-PCR plate combination was centrifuged (152/02 Sigma-Qiagen rotor of a 4–15 C benchtop centrifuge) for 4 min at RCF 840×g using the PCR plate as a collection plate. The purified DNA was used in the TaqMan assay for *X. fragariae*.

TaqMan-assay

The TaqMan assay used in the Direct, Bio- and Colony-Taqman tests was conducted according to Weller et al. (2007) with a few modifications as described in Kastelein et al. (2018). Samples were considered positive at a cycle threshold (Ct-value) of the curve describing the kinetics of the TaqMan reaction, lower than 34 and negative at a Ct-value higher than 35. Samples with Ct-values between 34 and 35 were also considered positive, but with a marginal value.

Data analysis

Basic data analysis (mathematical and graphic calculations) was done with the facilities of Microsoft Office Excel (Microsoft co., USA). Analysis of variance (ANOVA) was done with the facilities of Genstat (VSN International Ltd, UK). The data on upwind controls and the runs in which non-inoculated control plants were trimmed to check for the presence of background *X. fragariae* populations were not included in the analysis.

To examine the possible interrelationships between densities of *X. fragariae* cfu in the air and the densities of fine as well as of coarse particles in the trimming clouds, separate XY scatter plots were made for each of the two categories of particles with data sets for the two experiments in which ALS-affected plants with dry leaves were trimmed and for the two experiments in which plants with wetted leaves were trimmed. The log-transformed densities of *X. fragariae* cfu were plotted along the X-axis and the log-transformed averages of the densities of the two accompanying Dylos DC1700 air quality monitors at both sides of the Coriolis air samplers were plotted along the Y-axis. The R^2-values of the linear trendlines fitted to the data were used to represent the degree of association between the densities of *X. fragariae* cfu and the densities of the two categories of particles. Graphic calculations were also used to estimate the dispersion ranges of the two categories of particles. For each event in the course of which ALS-affected plants were trimmed, an XY scatter plot was made in which the log-transformed density data were plotted along the Y-axis and the accompanying untransformed distances (m) downwind from the strawberry plants were plotted along the X-axis. The distance at which the result of the linear trendline equations fitted to the data approached a density of 1 particle m^{-3} was considered as estimate of the dispersion range.

ANOVA was used to test for possible differences in logarithmic-transformed source strengths of the plants used in the trimming experiments. The effects of distance from ALS-affected strawberry plants and the foliar state (dry or wetted leaves) of the plants was analysed, on the untransformed data on the amounts of wind-dispersed haulm fragments settled down on the ground, and the logarithmic-transformed densities of *X. fragariae* cfu in windblown haulm fragments and in the air. ANOVA was also used to analyse the

effects of distance from the strawberry plants and their foliar state on the logarithmic-transformed densities of fine and coarse particles in the trimming clouds. Furthermore, ANOVA was used to analyse the effect of the foliar state of the trimmed plants and the experimental day on the untransformed dispersion ranges of fine and coarse particles in the trimming clouds.

Results

Source strength

In the four experiments carried out, only inoculated plants were examined for *X. fragariae* infections. Pathogen densities in the leaves of the individual plants tested ranged from 2.2×10^5 to 3.3×10^{10} cfu g^{-1}, with an average of 1.8×10^9 cfu g^{-1}. Analysis of variance (ANOVA) indicated that within each experiment the differences in *X. fragariae* density in the leaves of the plants used during the three trimming runs with ALS-affected strawberry plants were not statistically significant, but in the main the source strength of the plants used in the 1st experiment was lower ($P=0.038$) compared to the source strength of the plants used in the three other experiments (Table 1).

Dispersal via windblown haulm fragments

During trimming of the strawberry plants, the rear of the rotary mower ejected leaf and other material into the air, leading to the downwind dissemination of haulm fragments to the nearby surroundings. Up to approximately 4 m downwind from the row of source plants, leaf fragments could easily be detected with the unaided eye on the grass of the experimental plot. However, the sizes and amounts of haulm fragments settled down on the ground at the sampling spots depicted in Fig. 1 decreased rapidly with the distance to the strawberry plants from 8 – 16 g m^{-2} on the paving stones between the strawberry plants trimmed with dry leaves and 28– 48 g m^{-2} plants trimmed with wetted leaves, 3 – 20 g m^{-2} (plants trimmed with dry leaves) and 7 – 9 g m^{-2} (plants trimmed with wetted leaves) in the trays at 1.3 m to small amounts of 0 – 9 mg m^{-2} (plants trimmed with dry leaves as well as plants trimmed with wetted leaves) at 5 m downwind from the plants. At 10 m downwind from the strawberry plants, visible haulm fragments were never detected.

The amount of haulm fragments settled down between the strawberry plants after trimming plants with dry leaves was significantly smaller than after trimming plants with wetted leaves (Table 2). On the other hand, the differences in amounts of haulm fragments of plants trimmed with dry leaves and plants

Table 1 Source strength of the strawberry plants used in the sessions in which *Xanthomonas fragariae* inoculated leaves were trimmed

		Trimming run[1]								
	Day	2 Xf1			3 Xf2			4 Xf3		
Experiment	number	Average[2]	± st. dev.		Average	± st. dev.		Average	± st. dev.	
1	302	1.1×10^9	$\pm1.7\times10^9$	ab	5.6×10^8	$\pm1.0\times10^9$	ab	5.9×10^8	$\pm1.7\times10^9$	a
2	309	8.1×10^8	$\pm1.2\times10^9$	b	6.8×10^8	$\pm1.2\times10^9$	b	9.3×10^8	$\pm1.1\times10^9$	b
3	313	9.7×10^8	$\pm1.8\times10^9$	b	9.8×10^9	$\pm1.7\times10^{10}$	b	5.9×10^8	$\pm1.7\times10^9$	b
4	316	1.1×10^9	$\pm1.7\times10^9$	b	2.0×10^9	$\pm1.6\times10^9$	b	2.2×10^9	$\pm2.1\times10^9$	b

[1]1st (run 2 Xf1), 2nd (run 3 Xf2) and 3rd (run 4 Xf3) session of an experiment in which the leaves of *X. fragariae* inoculated plants were trimmed

[2]The average and standard deviation of the densities (cfu g^{-1}) of *X. fragariae* in the leaves of four plants per trimming run sampled several minutes before the concerned trimming run. The characters in the columns after the standard deviations describe the results of a two-factor analysis of variance (ANOVA) with the logarithmic transformed densities of the pathogen in the leaves of the four plants sampled per trimming run. In the ANOVA the batch of plants trimmed during an experiment was the primary treatment and the trimming run the secondary treatment. Average densities followed by identical characters (a, b) do not differ significantly (Duncan's multiple range test, $P=0.05$)

Table 2 Amounts of wind dispersed haulm fragments settled down on the ground at various distances downwind from trimmed strawberry plants showing symptoms of angular leaf spot

Distance from trimmed plants	Foliar state during leaf trimming					
	Dry leaves[1]			Wetted leaves		
	Average	± st. dev.		Average	± st. dev.	
0 m	12.2[2]	± 4.8	a	37.7	± 18.9	b
1.3 m	11.6[3]	± 12.3	a	7.7	± 1.6	a
5 m	< 0.1[3]	± < 0.1	a	< 0.1	± < 0.1	a
10 m	0.0[3]	± 0.0	a	0.0	± 0.0	a

[1]Condition of the leaf surface of plants coming straight from the screenhouse, plants with wetted leaves were sprinkled a few minutes before each trimming run using a garden spray gun to imitate trimming after a rain shower

[2]The average and standard deviation of the amounts (g m^{-2}) of the haulm fragments collected between the trimmed strawberry plants several minutes after each of the three leaf trimming sessions of the two experiments with trimming leaves with either dry or wetted leaves

[3]The average and standard deviation of the pooled amounts (g m^{-2}) of wind blown haulm fragments (one per experiment) collected in the field downwind from the trimmed plants during the two experiments with trimming leaves with either dry or wetted leaves. Averages and standard deviations of < 0.1 g m^{-2} indicate that only amounts of a few mg m^{-2} were collected at that distance. The characters in the columns after the standard deviations describe the results of a two-factor analysis of variance (ANOVA) with the averages of the amount of material in the three samples of haulm fragments per experiment collected at 0 mand the amount of material in the pooled samples of wind blown haulm fragments collected in the field at 1.3, 5 and 10 mdownwind from the trimmed plants. In the ANOVA the distance downwind from the plants was the primary treatment and the foliar state of these plants the secondary treatment. Average amounts of haulm fragments followed by identical characters (a, b) do not differ significantly (Duncan's multiple range test, $P = 0.05$)

trimmed with wetted leaves at 1.3 m and at 5 m distance downwind were statistically not significant. The ANOVA indicated a connection of the amount of haulm fragments that settled down on the ground with distance from the trimmed strawberry plants ($P < 0.003$) and not with their foliar state (dry or wetted; $P = 0.152$).

After dilution plating of the extracts of the haulm fragments collected between the non-inoculated control plants of all four trimming experiments and the extracts from haulm fragments of control plants of the 1st (trimming dry leaves) and 2nd (trimming wetted leaves) experiment collected in the trays at 1.3 m, no colonies of *X. fragariae* were detected on R2AGRC plates. However, the results of the Bio-TaqMan tests done on these plates were positive. The results of the Direct TaqMan tests done on these extracts confirmed the results of the Bio-TaqMan tests. In the 3rd (trimming dry leaves) and 4th (trimming wetted leaves) experiment colonies of *X. fragariae* were detected (in densities of 3.1×10^2 and 6.2×10^3 cfu m^{-2}, respectively) in samples of the control plants collected at 1.3 m. The presence of the pathogen was confirmed by Direct TaqMan and Colony-TaqMan tests (results presented in Table S2).

X. fragariae was detected (by Direct TaqMan tests as well as visually on R2AGRC plates and subsequent Colony-TaqMan tests) in all samples of wind-blown haulm fragments collected after trimming ALS-diseased plants. Irrespective of the foliar state of the plants during trimming, the numbers of cfu per surface area sharply decreased with increasing distance downwind from the trimmed strawberry plants (Table 3). The ANOVA indicated that the haulm fragment associated *X. fragariae* density on the ground was connected with the distance from the trimmed strawberry plants ($P < 0.002$) and not with their foliar state ($P = 0.964$).

Presence of *X. fragariae* in the air

The samples collected with a Coriolis Micro air sampler shortly before trimming the non-inoculated control plants of all four trimming experiments at 4 m upwind, were negative in the Direct TaqMan test and no colonies of *X. fragariae* were detected in the plating test. The results of the Bio-TaqMan tests done on the R2AGRC plates were also negative (results presented in Table S3). After trimming the non-inoculated controls of the 1st, 3rd and 4th

Table 3 Densities of haulm fragment associated *Xanthomonas fragariae* (cfu m^{-2}) deposited on the ground at various distances downwind from trimmed strawberry plants

Distance from trimmed plants	Foliar state during leaf trimming					
	Dry leaves[1]			Wetted leaves		
	Average[2]	± st. dev.		Average	± st. dev.	
0 m	1.7×10^{12}	$\pm9.5\times10^{11}$	b	3.7×10^{12}	$\pm1.9\times10^{12}$	b
1.3 m	3.0×10^{9}	$\pm1.6\times10^{9}$	b	4.4×10^{9}	$\pm4.1\times10^{9}$	b
5 m	2.7×10^{5}	$\pm3.9\times10^{5}$	a	1.9×10^{4}	$\pm2.7\times10^{4}$	a

[1]Condition of the leaf surface of plants coming straight from the screenhouse, plants with wetted leaves were sprinkled a few minutes before each trimming run using a garden spray gun to imitate trimming after a rain shower

[2]The average and standard deviation of the densities (cfu m^{-2}) of *X. fragariae* in the samples of haulm fragments collected in the field during the two experiments with trimming leaves with either dry or wetted leaves. At 0 m distance the figures relate to six samples (three per experiment) from between the trimmed strawberry plants. At 1.3 and 5 m distance the figures are derived from the pooled samples (one per experiment) of wind dispersed fragments downwind from the trimmed plants. The characters in the columns after the standard deviations describe the results of a two- factor analysis of variance (ANOVA) with the logarithmic transformed averages of the densities of the pathogen in the three samples of haulm fragments per experiment collected at 0 m and the logarithmic transformed densities of the pathogen derived from the pooled samples of wind dispersed haulm fragments collected in the field at 1.3 and 5 m downwind from the trimmed plants. In the ANOVA the distance downwind from the plants was the primary treatment and the foliar state of these plants the secondary treatment. Averages followed by identical characters (a, b) do not differ significantly (Duncan's multiple range test, $P=0.05$)

experiment the air samples collected at 1.3 and 10 m downwind from the source plants were *X. fragariae* negative in the Direct TaqMan, plating and Bio-TaqMan tests. On the other hand, the air sample of the non-inoculated control of the 2nd experiment collected at 1.3 m was positive in the Direct TaqMan test with a marginal Ct-value of 34.9. Although no colonies of *X. fragariae* were detected after plating that air sample, the Bio-TaqMan test was *X. fragariae* positive (Ct-value: 19.9). In the air sample of the 2nd experiment collected at 10 m downwind from the control plants the pathogen was detected in a density of 2.0×10^{2} cfu m^{-3} in the plating test. The Direct TaqMan test was also *X. fragariae* positive. The plating result was confirmed by the Colony-TaqMan test.

With the air samples collected at 1.3, 5, 10 and 25 m downwind during trimming ALS-diseased plants, varying Direct TaqMan test results were obtained. On the whole, the Ct-values were high and only 56% of the samples were *X. fragariae* positive. However, in the plating tests *X. fragariae* was detected in all air samples (all plating results were confirmed by Colony-TaqMan tests; Table S3). Irrespective of the foliar state of the trimmed plants a decrease was found in the density of the pathogen in the air at 47 cm above ground level with increasing distance downwind from the trimmed plants (Table 4). The ANOVA indicated a strong connection between *X. fragariae* density in the air and distance from the trimmed strawberry plants ($P=0.021$) and no connection between foliar state (dry or wetted) and pathogen density ($P=0.219$).

Generation of windblown aerosol particles

The data collected with the Dylos air quality monitors placed downwind from the strawberry plants showed that at 45 cmabove ground level trimming led to periods of on average 32 s with peaks in the densities of both fine and coarse particles in the air. The increment in densities of coarse particles in the trimming run air (the air associated with trimming strawberry plants) was on average higher than with fine particles. Peaks in the densities of fine and coarse particles, similar to those recorded by the air quality monitors placed downwind from plants, were not observed in the recordings of the air quality monitor placed 4 mupwind in any of trimming experiments.

For each air quality monitor placed downwind from the strawberry plants the background densities of fine and coarse particles in the air were subtracted from the particle densities in the air recorded during periods associated with trimming strawberry plants to estimate the densities of these particles in the trimming clouds at the distance linked with the monitor concerned. Irrespective of the foliar state

Table 4 Downwind decrease in the densities of *Xanthomonas fragariae* cfu m^{-3}in the air at 47 cm above ground level associated with trimming angular leaf spot diseased strawberry plants with dry or wetted leaves

Distance from trimmed plants	Foliar state during leaf trimming					
	Dry leaves[1]			Wetted leaves		
	Average[2]	± st. dev		Average	± st. dev	
1.3 m	6.8×10^5	$\pm9.4\times10^5$	b	3.0×10^5	$\pm2.7\times10^5$	b
5 m	1.3×10^5	$\pm1.6\times10^5$	b	1.8×10^5	$\pm2.5\times10^5$	b
10 m	3.4×10^4	$\pm5.6\times10^4$	ab	1.3×10^5	$\pm1.8\times10^5$	b
25 m	4.8×10^2	$\pm4.6\times10^2$	a	4.4×10^3	$\pm3.2\times10^3$	ab

[1]Condition of the leaf surface of plants coming straight from the screenhouse, plants with wetted leaves were sprinkled a few minutes before each trimming run using a garden spray gun to imitate trimming after a rain shower

[2]The average and standard deviation of the densities (cfu m^{-3}) of *X. fragariae* in the air samples collected in the field downwind from the plants during trimming either dry or wetted leaves. At 1.3, 5 and 25 m distance the figures relate to one sample per experiment. At 10 m distance the figures relate to six samples (three per experiment). The characters in the columns after the standard deviations describe the results of a two-factor analysis of variance (ANOVA) with the logarithmic transformed average of the densities of the pathogen in the three air samples per experiment collected at 10 m and the logarithmic transformed densities of the pathogen in the single air sample per distance and experiment collected at 1.3, 25 and 5 m downwind from the trimmed plants. In the ANOVA the distance downwind from the plants was the primary treatment and the foliar state of these plants the secondary treatment. Averages followed by identical characters (a, b) do not differ significantly (Duncan's multiple range test, $P=0.05$)

(dry or wetted) of the trimmed plants the density of both fine and coarse particles in the trimming clouds at 45 cm above ground level decreased with increasing distance downwind from the trimmed plants. Table 5 describes the decline in fine and course particles in the trimming clouds for the two experiments with trimming plants with dry leaves and for the two experiments with trimming plants with wetted leaves. The ANOVA indicated a connection of the density of fine particles with the distance from the trimmed strawberry plants ($P<0.001$) and not with the foliar state (dry or wet) of the plants ($P=0.223$), as well as not with the line parallel to the preponderant wind direction along which the Dylos air quality monitors were placed ($P=0.700$). For the density of coarse particles the statistical analyses indicated a connection with the distance ($P<0.001$) and their foliar state ($P=0.005$), but not with the line parallel to the preponderant wind-direction along which the Dylos air quality monitors were placed ($P=0.199$).

Interrelationships between densities of *X. fragariae* cfu in the air and fine as well as coarse aerosol particles in the trimming clouds

The simultaneous downwind declines of *X. fragariae* cfu, fine particles and coarse particles in the trimming clouds of ALS-diseased strawberry plants suggest relationships between *X. fragariae* cfu and these particles. In Fig. 2, left XY scatter plot, linear trendlines for strawberry plants with dry and wetted leaves illustrate the interrelationships between log-transformed averages of the densities of fine particles derived from the recordings of the pairs of Dylos air quality monitors on either side of the Coriolis air samplers and the densities of *X. fragariae* cfu. The linear trendlines in Fig. 2, right XY scatter plot, do this for the coarse particles. The equations describing the linear trendlines and the corresponding R^2-values.

are presented in Table 6. The trendline equations describing the interrelationships between the densities of *X. fragariae* cfu and the densities of coarse particles had a better fit to the observed data than the trendline equations describing the interrelationships between the densities of *X. fragariae* cfu and the densities of fine particles.

Estimated dispersion ranges of fine and coarse aerosol particles in trimming clouds

The data on the densities of the fine and coarse particles in trimming clouds at different distances downwind from the trimmed strawberry plants were plotted against the corresponding distances of origin. The equations describing the linear trendlines fitted to the data and the dispersion distances

Table 5 Downwind decrease in the densities of fine and coarse particles (N m^{-3}) in the clouds of matter ejected into the air during trimming angular leaf spot diseased strawberry plants with dry or wetted leaves

Aerosol particles[2]	Downwind distance	Foliar state during leaf trimming					
		Dry leaves[1]			Wetted leaves		
		Average[3]	± st. dev.		Average	± st. dev.	
Fine	1.3 m	3.7×10^{7}	$\pm1.4\times10^{7}$	d	4.5×10^{7}	$\pm1.9\times10^{7}$	d
Fine	5 m	2.8×10^{7}	$\pm7.7\times10^{6}$	cd	3.8×10^{7}	$\pm2.2\times10^{7}$	cd
Fine	10 m	1.4×10^{7}	$\pm7.8\times10^{6}$	bcd	1.5×10^{7}	$\pm1.4\times10^{7}$	bcd
Fine	25 m	4.4×10^{6}	$\pm3.5\times10^{6}$	abcd	2.3×10^{6}	$\pm3.4\times10^{6}$	abc
Fine	50 m	1.5×10^{6}	$\pm1.6\times10^{6}$	a	1.5×10^{6}	$\pm2.0\times10^{6}$	ab
Coarse	1.3 m	7.9×10^{6}	$\pm3.4\times10^{6}$	e	7.8×10^{6}	$\pm4.0\times10^{6}$	e
Coarse	5 m	5.7×10^{6}	$\pm2.1\times10^{6}$	de	7.4×10^{6}	$\pm5.0\times10^{6}$	e
Coarse	10 m	2.9×10^{6}	$\pm1.3\times10^{6}$	cde	2.3×10^{6}	$\pm2.3\times10^{6}$	abcd
Coarse	25 m	7.1×10^{5}	$\pm5.2\times10^{5}$	ab	3.6×10^{5}	$\pm4.5\times10^{5}$	a
Coarse	50 m	9.3×10^{5}	$\pm6.5\times10^{5}$	abc	4.3×10^{5}	$\pm2.8\times10^{5}$	ab

[1]Condition of the leaf surface of plants coming straight from the screenhouse, plants with wetted leaves were sprinkled a few minutes before each trimming run using a garden spray gun to imitate trimming after a rain shower

[2]Fine: microscopic particles of size distribution 0.5 – 2.5 µm. Coarse: microscopic particles of size distribution 2.5 – 10 µm

[3]The average and standard deviation of the densities (N m^{-3}) of fine and coarse aerosol particles in the clouds of matter ejected into the air during the two experiments with trimming leaves with either dry or wetted leaves. At 1.3, 5, 10 and 25 m distance the figures relate to six recordings per experiment and at 50 m distance to three recordings per experiment. The characters in the five rows of fine particles after the standard deviations describe the results of a three-factor analysis of variance (ANOVA) with the logarithmic transformed densities (six per distance and experiment) of fine particles in the trimming clouds derived from the recordings at 1.3, 5, 10 and 25 m downwind from the trimmed plants of the air quality monitors along the two lines running parallel to the preponderant wind direction and the logarithmic transformed densities (three per experiment) of fine particles in the trimming cloud derived from the recordings of the air quality monitor at 50 m downwind from these plants. In the ANOVA the distance downwind from the plants was the primary treatment, the foliar state of these plants the secondary treatment and the line along which the data were collected the third treatment. Average densities of fine particles followed by identical characters (a- d) do not differ significantly (Duncan's multiple range test, $P=0.05$). The characters in the five rows of coarse particles after the standard deviations describe the results of a similar ANOVA with the logarithmic transformed densities of coarse particles in the trimming clouds. Average densities of coarse particles followed by identical characters (a- e) do not differ significantly

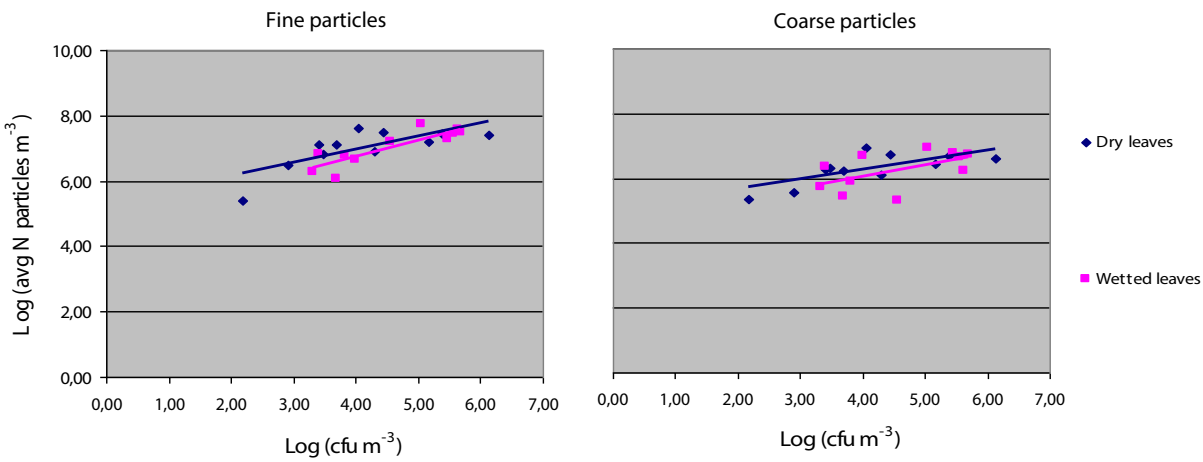

Fig. 2 Interrelationships between the density of fine (left) and coarse (right) particles in the trimming clouds and the density of *X. fragariae* colony forming units (cfu) present in the air connected with trimming angular leaf spot diseased strawberry plants with dry or wetted leaves. Solid lines represent linear trendlines

Table 6 Equations of the linear trendlines describing the interrelationships between the estimated density of fine[1] and coarse[2] particles and the density of *X. fragariae* cfu present in the air connected with trimming angular leaf spot diseased strawberry plants with dry or wetted leaves

Units	Trimming plants with dry leaves[3]		Trimming plants with wetted leaves	
	Trendline Eq. [4]	R^2-value	Trendline equation	R^2-value
Fine particles	$y = 5.167 + 0.4397x$	0.54	$y = 5.247 + 0.4011x$	0.54
Coarse particles	$y = 5.0454 + 0.3217x$	0.60	$y = 4.3746 + 0.4220x$	0.64

[1]Microscopic particles of size distribution 0.5 – 2.5 µm

[2]Microscopic particles of size distribution 2.5 – 10 µm

[3]Condition of the leaf surface of plants coming straight from the screenhouse, plants with wetted leaves were sprinkled a few minutes before each trimming run using a garden spray gun to imitate trimming after a rain shower

[4]In the trendline equation *y* represents the logarithmic transformed averages of the two densities ($N_{particles}$ m^{-3}) of the fine particles or the coarse particles recorded by the Dylos air quality monitors at both sides of the Coriolis air samplers and *x* the accompanying log-transformed densities of *X. fragariae* cfu ($N_{X.\ fragariae\ cfu}$ m^{-3})

deduced from these equations are presented in Table S4. For the fine particles the trendline equations produced dispersion ranges between 46 and 392 m (plants with dry leaves) and between 59 and 344 m (plants with wetted leaves). The ANOVA indicated a connection with the experimental day ($P = 0.017$), but no connection with the foliar state of the plants ($P = 0.192$) and the dispersion range of fine particles (Table 7). The equations describing the decline of the densities of the coarse particles produced dispersion distances that varied from 138 to 296 m for plants with dry leaves and from 161 to 280 m for plants with wetted leaves. The ANOVA indicated no connection with the experimental day ($P = 0.236$) and also no connection with the foliar state of the plants ($P = 0.593$) and the dispersion range of coarse particles.

Table 7 Effect of the foliar state during trimming strawberry plants on propagation beds and experimental day on the estimated dispersion ranges of fine and coarse aerosol particles in the trimming clouds

Aerosol particles[2]	Foliar state during leaf trimming							
	Dry leaves[1]				Wetted leaves			
	Experiment	Average[3]	± st. dev.		Experiment	Average	± st. dev.	
Fine	1	79.7 m	± 44.4 m	a	2	95.0 m	± 34.2 m	ab
Fine	3	343.3 m	± 42.7 m	c	4	200.7 m	± 124.2 m	bc
Coarse	1	185.0 m	± 42.5 m	a	2	226.3 m	± 27.0 m	a
Coarse	3	274.3 m	± 35.0 m	a	4	201.3 m	± 68.1 m	a

[1]Condition of the leaf surface of plants coming straight from the screenhouse, plants with wetted leaves were sprinkled a few minutes before each trimming run using a garden spray gun to imitate trimming after a rain shower

[2]Fine: microscopic particles of size distribution 0.5 – 2.5 µm. Coarse: microscopic particles of size distribution 2.5 – 10 µm

[3]The average and standard deviation of the estimated dispersion ranges of fine and coarse aerosol particles in the clouds of matter ejected into the air during the two experiments with trimming leaves with either dry or wetted leaves. The characters in the two rows of fine particles after the standard deviations describe the results of a two-factor analysis of variance (ANOVA) with the untransformed dispersion ranges (three per experiment) of fine particles in the trimming clouds calculated by means of the trendline equations of XY scatter plots in which the log-transformed density data were plotted along the Y-axis and the accompanying untransformed distances (m) downwind from the strawberry plants were plotted along the X-axis. In the ANOVA the foliar state of the plants was the primary treatment and the experiment the secondary treatment. Average densities of fine particles followed by identical characters (a- c) do not differ significantly (Duncan's multiple range test, $P = 0.05$). The characters in the two rows of coarse particles after the standard deviations describe the results of a similar ANOVA with the untransformed dispersion ranges of coarse aerosol particles. Average dispersion ranges of coarse particles followed by identical characters do not differ significantly

Discussion

In previous leaf trimming experiments (Kastelein et al., 2018), which demonstrated that in strawberry propagation crops a rotary mower can cause dissemination of *X. fragariae* from ALS-diseased plants to healthy plants within these crops, indications were found for the aerial dispersion of the pathogen to neighbouring beds. The present study confirmed that *X. fragariae* can be dispersed by air. During trimming ALS-diseased plants, leaf fragments and other material accommodating the pathogen were ejected into the air. Haulm fragments settled down on the ground a few metres downwind of the strawberry plants, microscopic aerosol particles were detected in the air and *X. fragariae* was recovered from air samples collected at distances up to 25 mdownwind from the trimmed crop. The role of mowing machines in the aerial dispersion of plant pathogenic bacteria has been shown before for *Pectobacterium carotovorum* during the pulverization of the haulms of potato crops prior to the harvest of the tubers (Pérombelon et al., 1979) and for *Xanthomonas vesicatoria* during topping of tomato seedlings cultivated in the field for transplant production (McInnes et al., 1988).

Despite the absence of symptoms of ALS disease in the non-inoculated strawberry tray plants, *X. fragariae* was recovered from air samples (2nd experiment) and windblown haulm fragments (3rd and 4th experiment) collected after trimming these negative control plants. As the plants were obtained from a recognized propagator, this finding was unexpected. Probably the non-inoculated plants became contaminated as a result of wind-driven aerosols formed on rainy days (EPPO, 1997) at the time they were kept in a screenhouse compartment next to the compartment with inoculated plants.

The discrepancy in the results of plating tests for one side and the results of the Direct TaqMan and Bio-TaqMan tests for another, done on windblown haulm fragments of control plants, exposes the difficulty recovering low numbers of the slow-growing *X. fragariae* (Kennedy & King, 1962a) due to the absence of a suitable selective medium. On culture media that support the growth of *X. fragariae*, rapidly growing saprophytic bacteria often prevent growth of the pathogen (Hildebrand et al., 1967; Rat, 1993). In the present study a Rifampicin resistant strain and glycine amended R2A Agar, a culture medium enabling viable counts of low-density suspensions of the pathogen (Kastelein et al., 2014) to which Rifampicin and cycloheximide was added to improve selectivity, were used. Despite the addition of antibiotics, non-target bacteria, yeasts and fungi grew on R2AGRC plates seeded with undiluted or 100×diluted plant extract. These background micro-organisms very likely interfered with the growth of cells of *X. fragariae* they outnumbered.

During trimming strawberry tray plants the bulk of the detached leaf and petiole fragments settled down between the plants. However, some of these haulm fragments were ejected into the air through the open back side of the mower, taken up by the wind and deposited on the ground downwind of the plants. In case of the plants with wetted leaves, the amount of haulm fragments that settled down between the trimmed plants was larger than that of the plants with dry leaves, probably the layer of water on the wetted leaves made haulm fragments stick together to lumps of plant material which were, as a result of their weight, less subject to being ejected into the air through the open back side of the mower. After trimming ALS-affected plants *X. fragariae* was recovered from all samples of haulm fragments collected in the field. This clearly demonstrates that the pathogen can be dispersed via strawberry haulm fragments over short distances. In the present study, when light and moderate breezes were blowing, dispersal of leaf fragments of both dry plants and wetted plants was limited to 4 – 5 m. However, it cannot be ruled out that during more powerful winds longer distances can be covered. Another aspect to be taken into account is that after initial deposition the leaf fragments might be picked up by the wind again and thus once more be spread out. When symptomatic leaves are still attached to ALS-affected plants, biofilms with cells of *X. fragariae* can ooze out of the leaf tissues through stomatal openings in the lower epidermis and spread out on the abaxial leaf surface (Allan-Wojtas et al., 2010). As *X. fragariae* can overwinter in Minnesota (USA) in leaves (suspended in the air in nylon bags and buried in pots with field soil) stored outside (Kennedy & King, 1962b) it can not be ruled out that biofilms with viable cells of the pathogen will still be available on the surface of the scattered leaf fragments. These biofilms of *X. fragariae* on the leaf fragments will as good as certain be susceptible to water-splash dispersal. Therefore, water-splashes occurring

during rainfall or overhead irrigation events (Van der Wolf et al., 2017) during the first weeks after trimming the strawberry plants may be responsible for secondary infections with *X. fragariae*.

Simultaneously with the aerial dispersion of *X. fragariae* via detached macroscopic haulm fragments ejected into the air during trimming strawberry plants, also the possibility of dispersion of the pathogen via microscopic particles ejected into the air during this operation was investigated. The short periods, matching the points in time and duration of the trimming runs, with peaks in the densities of 0.5 – 10 µm sized particles in the air recorded by Dylos air quality monitors downwind from the trimmed strawberry plants, and the absence of peaks in the densities of these particles in the recordings of the upwind air quality monitors, irrefutably demonstrate that microscopic particles were ejected into the air during trimming strawberry plants. The absence of *X. fragariae* in the air samples collected by Coriolis air samplers before commencement of the experiments at 4 m upwind of the experimental plot and the presence of the pathogen in all air samples collected downwind from the trimmed ALS-affected strawberry plants indicate that these plants are the source of *X. fragariae* in the air. Consequently, the results of the bacteriological examination of the air samples collected by Coriolis air samplers downwind from trimmed ALS-affected strawberry plants and the increments in densities of fine and coarse particles recorded during trimming strawberry plants by the accompanying air quality monitors at both sides of the Coriolis air samplers justify the assumption that the presence of *X. fragariae* in the air is somehow connected with these microscopic particles. In microscopic images of ALS-affected leaves, cells of *X. fragariae* measure 1.0 – 1.8 µm long and ≈0.5 µm wide (Allan-Wojtas et al., 2010). Because of their dimensions, cells of *X. fragariae*, once airborne, will be detected by the air quality monitors used in present study. Single bacterial cells and small agglomerates of a few bacterial and plant cells will be recorded as fine particles and part of the more complex agglomerates as coarse particles.

The densities of *X. fragariae* cfu, fine and coarse particles in the trimming clouds declined with increasing distance travelled downwind from the trimmed strawberry plants. These declines in densities bear a strong resemblance to the declines in densities of spores described by Gregory (1945) for the aerial dispersion of clouds of fungal spores. Immediately after the release of a cloud of fungal spores the individual spores are exposed to various forces such as gravity, wind-currents and electrostatic forces (Lighthart, 1994). During their glide through the air the spores are coming down as a result of gravity as well as being dispersed in all directions by air turbulence, resulting in a continuous dilution of the spore cloud, while the expanding cloud is being carried along in the main direction of the wind. In clouds of fungal spores the individual spores are normally of uniform dimensions. In contrast, the trimming clouds in present experiments are likely made up of a variety of particles (such as single bacterial cells, droplets of sap with or without bacterial cells, solid agglomerates of bacterial cells, agglomerates of plant cells with or without bacteria, dust and smut particles of the exhaust fumes of the tractor) with a wide range of dimensions. As during flail mowing of cover crops (mixtures of graminaceous and leguminous plants) particles sized up to 100 µm were ejected into the air (Baker et al., 2005), it is plausible that during trimming the strawberry plants, in addition to the 0.5 – 10 µm sized particles, also particles with diameters between 10 and 100 µm were ejected into the air.

In experiments on the splash dispersal of *X. fragariae* from ALS-affected plants during overhead irrigation at moderate wind, the pathogen was spread over a distance of at least 4 m (Van der Wolf et al., 2017). In water drops dripping from irrigated symptomatic leaves the pathogen was found to be present in high numbers of cfu (unpublished observations). In view of these observations it was supposed that during trimming plants with wetted leaves more cells of *X. fragariae* would be ejected into the air compared to trimming plants with dry leaves. However, at all distances sampled the averages of the densities of *X. fragariae* cfu, fine particles and coarse particles of the two treatments (dry or wetted leaves) were not significantly different. This is most probably due to the differences in the height of the densities found between the two experiments per foliar state (dry or wetted) and the overlapping of the ranges of the densities found after trimming plants with dry leaves and the ranges of densities found after trimming plants with wetted leaves. This high variation in the height of the densities can possibly be explained by the fact that each experiment was done on a different day with

different atmospheric conditions, differently influencing the paths travelled by the trimming clouds with respect to the positions of the air samplers and air quality monitors in the field and, if applicable, the drying of the leaves of wetted plants.

The simultaneous downwind declines in the densities of *X. fragariae* cfu, fine particles and coarse particles in the trimming clouds of ALS-diseased strawberry plants suggest certain connections between *X. fragariae* cfu and the two categories of aerosol particles. Linear regression analysis supported this, as for both fine and coarse particles medium to strong associations between the densities of *X. fragariae* cfu and the densities of these particles in the trimming clouds of both plants with dry leaves and plants with wetted leaves were found. As is shown in Tables 4 (densities of *X. fragariae* cfu) and 5 (densities of fine and coarse particles), the level of the densities of *X. fragariae* cfu was considerably lower than those of the fine and the coarse particles in the trimming clouds. These differences are almost certainly due to the methods applied for assessing the densities of *X. fragariae* cfu on one side and the densities of the fine and coarse particles for the other. During sampling the air with Coriolis air samplers the whole range of sizes of aerosol particles (diameters between ≈0.1 and ≈1000 µm; Lighthart & Mohr, 1994) is collected. The densities of *X. fragariae* cfu only relate to the presence of viable cells of the pathogen in the sample and provide no information on the size or nature (cells of *X. fragariae* incorporated in microscopic pieces of strawberry plant tissues, free agglomerates of several *X. fragariae* cells or single cells of the pathogen) of the particles of origin. Dylos air quality monitors only record the densities of particles of the size distributions 0.5 – 10 µm (fine and coarse particles together) and 2.5 – 10 µm (only coarse particles) in the air and does not provide information on the nature (liquid droplets, solid particles, plant debris, microorganism or pollen grain) or quality (dead or alive) of these particles. The densities of the fine and coarse particles in the trimming clouds relate to the rise in the densities of these particles as a result of trimming strawberry plants and does not provide information on the amount of aerosol particles ˃10 µm ejected into the air during that event. The medium to strong associations between the densities of *X. fragariae* cfu and the densities of fine and coarse particles in the trimming clouds make it more than reasonable that at least part of the *X. fragariae* cfu contribute to the populations of fine and coarse particles in the trimming clouds.

A better insight in the dispersion range of *X. fragariae* aerosols may be of use for the management of ALS disease. However, the limited data on the spatial decrease of the densities of *X. fragariae* cfu in the air (on each experimental day one sample at 1.3, 5 and 25 mdistance, obtained during different trimming runs) did not permit estimating the dispersion range of the pathogen. On the other hand, the availability of extensive sets of data on the spatial decrease of the densities of fine and coarse particles in every trimming cloud of an experimental day made it possible to obtain mathematical equations required to calculate dispersion distances for each trimming run. In the present study, when light and moderate breezes were blowing and the sky was for the most part overcast, the estimated dispersion distance for the particles of size distribution 0.5 – 10 µm was calculated to be between 46 and 392 m. The variability in the estimated dispersion distances may be connected with differences in the atmospheric conditions (deviations in wind-speed, deviations from the preponderant wind-direction) during the three trimming runs of an experiment and with differences in the atmospheric conditions during the four experimental days. As the dispersion of aerosols is influenced by wind-speed and turbulence of the air (Gregory, 1945) it is quite possible that under weather conditions with higher wind-speeds and more gusts of wind the fine and coarse particles ejected into the air during trimming strawberry plants will travel longer distances. It is plausible that, in addition to the part of the *X. fragariae* cfu linked with partictes of size distribution 0.5 – 10 µm, another part of the *X. fragariae* cfu is linked with larger aerosol particles. As air particles larger than about 50 µm in diameter have sufficient mass to cause them to sediment rapidly owing to gravity while fine and coarse particles sediment more slowly (Wickman, 1994), fine and coarse particles of the trimming cloud can travel longer distances than the larger particles containing cells of *X. fragariae*. For that reason the dispersion distances worked out for the fine and coarse particles can also be looked upon as plausible estimations of the distances *X. fragariae* aerosols can travel.

The present study shows that during trimming ALS-diseased plants haulm fragments and 0.5 – 10 µm sized aerosol particles are ejected into the air and that *X. fragariae* can be recovered from air samples. Calculations based on the data of the spatial decrease of

aerosol particles in trimming clouds indicate that, under weather conditions similar to those which occurred during the present study, these particles can be dispersed over distances between 46 and 392 m. The medium to strong associations between the densities of *X. fragariae* cfu in the air and the densities of fine and coarse particles in the trimming clouds indicate that windblown *X. fragariae* cfu may travel similar distances. This implies that during trimming an ALS-affected propagation bed all strawberry plants growing within a distance of several hundreds of metres downwind from the ALS-affected plants may become contaminated by cells of *X. fragariae*. Whether this *X. fragariae* contamination will result in infections all over the dispersion area is not known. Anyhow, previous field studies showed that viable cells of *X. fragariae* could be recovered from windblown water droplets loaded with the pathogen up to a distance of 100 m from the source (Van der Wolf et al., 2017) and that such artificially created aerosols could infect strawberry plants at a distance of at least 10 m from the source (Van der Wolf et al., 2018). With these experiences in mind, management of ALS should include roguing of symptomatic plants in order to reduce inoculum pressure, restriction of the release of *X. fragariae* contaminated aerosols during trimming strawberry plants on propagation beds by equipping the mower with facilities that prevent sideward and backward spread of clipped haulm fragments and aerosols as well as maintaining broad distances between strawberry propagation beds for the production of certified waiting bed plants and beds for the production of propagation stock II plants.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Conflict of interest The corresponding author (Jan van der Wolf) has received research funding from the Dutch growers of strawberry planting material. All other authors herewith declare that they have no conflict of interest.

Human and animals studies This study does not contain studies with human participants or animals performed by any of the authors.

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