Populations dynamics of *Stemphylium vesicarium* causing brown spot of pear and *Stemphylium* spp. non-pathogenic in pear on plant residues in Dutch pear and apple orchards

Jürgen Köhl · Lia Groenenboom-de Haas ·
Ilse Houwers · Marcel Wenneker

Abstract *Stemphylium vesicarium* causes brown spot of pear. Pear-pathogenic isolates of *S. vesicarium* and *S. vesicarium* isolates not able to cause symptoms on pear leaves and fruits has been reported. *Stemphylium* spp. have a necrotrophic lifestyle and crop debris is an important niche for *Stemphylium* spp. populations to survive, grow and multiply. We hypothesized that density and dynamics of populations of non-pathogenic *S. vesicarium* have an impact on the occurrence and dynamics of pear-pathogenic *S. vesicarium* populations during its saprotrophic phase. A genus-specific Taqman PCR assay for the quantification of *Stemphylium* spp. and a Taqman PCR assay specific for the pear-pathogenic *S. vesicarium* populations was applied to approximately 2000 litter samples from Dutch orchards. Co-occurrence of both pear-pathogenic *S. vesicarium* and *Stemphylium* spp. regarded as non-pathogenic were found in approximately 25% of the samples from pear orchards collected in an earlier study on brown spot of pear. Fluctuations for both *Stemphylium* spp. and pear-pathogenic *S. vesicarium* show the same pattern. No cases were found were increased concentrations of *Stemphylium* spp. coincided with a decrease in concentrations of pear-pathogenic *S. vesicarium*. The hypothesis that high colonization by saprophytic *Stemphylium* spp. may outcompete pear-pathogenic populations can thus not be supported.

Introduction

Stemphylium vesicarium (teleomorph: *Pleospora herbarum*) is a fungal pathogen with a broad host range, affecting crops such as pear (Llorente & Montesinos, 2006), asparagus (Falloon et al., 1987), garlic (Prados-Ligero et al., 1998), and onion (Aveling & Snyman, 1993). Isolates obtained in an earlier study from asparagus and onion were non-pathogenic in pear (Köhl et al., 2009a). *Stemphylium botryosum* (teleomorph: *P. tarda*), has been described as a pathogen of asparagus (Leuprecht, 1990), alfalfa (Cowling et al., 1981) and spinach (Koike et al., 2001). For other *Stemphylium* species more specific host ranges have been described, e.g. *S. alfalfae* (teleomorph: *P. alfalfae*) has been reported as a pathogen of alfalfa (Chaisrisook et al., 1995), *S. eturmiunum* of apple, sweet cherry and tomato (Cong et al., 2022; Spadoni et al., 2020; Prencipe et al., 2021) and *S. beticola* has been

J. Köhl (✉) · L. G. Haas · I. Houwers · M. Wenneker
Wageningen Plant Research, Wageningen University & Research, Wageningen, Netherlands
e-mail: jurgen.kohl@wur.nl

reported as a pathogen of sugar beet (Hanse et al., 2015; Woudenberg et al., 2017).

Stemphylium spp. have a necrotrophic lifestyle and grow saprophytically in necrotic host tissues. Crop debris is an important niche for *Stemphylium* spp. populations to survive, grow and multiply. Colonized crop debris is the main inoculum source for *Stemphylium* epidemics in susceptible crops as shown for *S. vesicarium* in garlic (Prados-Ligero et al., 1998), asparagus (Hausbeck et al., 1999) and pear (Rossi et al., 2008). Potentially, *Stemphylium* spp. can also colonize senesced plant tissues of non-host plants. In pear, a differentiation between pear-pathogenic isolates of *S. vesicarium* and *S. vesicarium* isolates not able to infect and cause symptoms on pear leaves and fruits has been observed (Köhl et al., 2009a, 2009b). Pear-pathogenic *S. vesicarium* are also common on non-host plant residues in pear orchards. Dead weeds and grasses on the orchard floor colonized by *S. vesicarium* are a main source of ascospores and conidia of *S. vesicarium* at the onset of the epidemic and during the entire growing season of pear (Köhl et al., 2009a, 2013; Rossi et al., 2008). Recently, woody pear tissues have also been described as potential inoculum source (Cavina et al., 2024). Artificial exclusion of inoculum sources using ground cover, litter removal and application of antagonists such as *Trichoderma* spp. and *Bacillus subtilis* on plant residues on the orchard floor potentially reduce the disease pressure in the pear crop (De Jong & Heijne, 2008; Llorente et al., 2010, 2012; Moragrega et al., 2021).

Fungal and bacterial communities colonize leaf litter as the primary agents of decomposition with a rapid successional change depending on the stage of decomposition, substrate quality and enzymatic abilities of the decomposers. Particularly, fungi are considered the major contributors to early decomposition because they have the ability to produce specific enzymes and can access new substrates through hyphae (Bani et al., 2018). The natural decomposition processes by fungal and bacterial communities and competitive processes amongst community members include interactions with pathogen populations present in the litter. Microbiomes in crop residues can thus have impact on pathogen survival and microbiome modulation may result in disease prevention (Berg et al., 2021; Kerdraon et al., 2019). Members of the fungal class of *Dothideomycetes* include *Pleosporales*, the fungal order which *Stemphylium* belongs to,

are amongst the major dominating fungal orders on living leaves and senesced leaves during their early decomposition (Schneider et al., 2012; Voriskova & Baldrian, 2013). *Dothideomycetes* are important players in microbial litter decomposition processes, contributing to this function of the microbiome by the production of cellulases (Schneider et al., 2012).

A remarkable variation between Dutch pear orchards is reported with high losses due to brown spot in few orchards whereas no infestation is reported on the majority of pear orchards. Factors determining this variation of disease incidence and severity between individual orchards and seasons are still unknown. Saprophytic *S. vesicarium* isolates besides pear-pathogenic isolates are present in litter of pear leaves, as well as of weeds and grasses in Dutch pear orchards (Köhl et al., 2009a). Co-existing saprophytic *S. vesicarium* populations may competitively suppress pear-pathogenic *S. vesicarium* populations.

We hypothesized that densities and dynamics of populations of non-pathogenic *S. vesicarium* have an impact on the occurrence and dynamics of pear-pathogenic *S. vesicarium* populations during its saprotrophic phase. The objective of our study was to investigate levels and dynamics of substrate colonization by non-pear-pathogenic *Stemphylium* spp. populations and pear-pathogenic *S. vesicarium* populations in orchard litter and to detect possible competitive interactions between them. A genus-specific Taqman PCR assay for the quantification of *Stemphylium* spp. and a Taqman PCR assay specific for the pear-pathogenic *S. vesicarium* populations (Köhl et al., 2009b) were applied to approximately 2000 litter samples collected in earlier studies on fruit rot diseases in 2009–2012 in 20 pear orchards and 10 apple orchards (Köhl et al., 2013, 2018). The same samples have been analyzed for DNA of *Fibulorhizoctonia psychrophila* in a recent study (Köhl et al., 2023).

Material and methods

Litter samples and DNA extracts

Litter samples were collected in Dutch orchards during two studies on the role of necrotic host tissue as possible inoculum sources of brown spot of pear (2009–2011; 10 pear orchards; Köhl et al., 2013) or post-harvest fruit rots in pear and apple (2012;

10 pear orchards and 10 apple orchards; Köhl et al., 2018). Ten pear orchards with cv. Conference were selected in 2008 in different parts of The Netherlands where symptoms of brown spot had been observed on leaves and fruits. Samples of various necrotic plant residues and prunings were collected in August 2009. In two of the orchards plant residues were also sampled monthly from February 2009 to May 2011 to investigate the dynamics of *S. vesicarium* on necrotic plant residues. Sampling plots were 25-m long tree strips of two neighbouring pear rows and the grass alley between the two rows. Residues of fallen pear leaves, approximately 100 ml, were collected. From twigs pruned during the previous growing season, ten 5-cm segments were cut and collected. If present, residues of fallen pears or mummies on the floor were sampled. Dead leaves of grasses, approximately 100 ml, were collected in tree strips and grass alleys. Dead weeds were sampled in the tree strips.

Ten apple orchards and 10 pear orchards were selected in 2012 in the main fruit-growing areas of the Netherlands to investigate the role of litter as potential inoculum source for the fruit rot pathogens *N. alba*, *N. perennans*, *C. malorum* and *C. luteo-olivacea*. Samples of various necrotic plant residues and tree parts were collected in the 20 orchards in May 2012 as described above. In four apple orchards and four pear orchards, samples were taken monthly from May until September and in December 2012. Detailed information on sampling can be found in Köhl et al. (2013) and Köhl et al. (2018).

Samples were stored in plastic bags at −18 °C. After storage, frozen samples were shredded. Subsamples of litter samples were freeze-dried, milled in a laboratory mill with a 1-mm mesh sieve to fine powders and stored at −20 °C in 96-well microtiter plates. DNA was extracted using the DNeasy 96 plant kit (Qiagen) (samples collected in 2009–2011) or the sbeadex maxi plant kit (samples collected in 2012) with several modifications and DNA extracts were stored at −20 °C. Detailed information on the origin of litter samples, protocols for sampling and sample processing including DNA extraction protocols and results for quantification of DNA of pear-pathogenic *S. vesicarium* and *N. alba*, *N. perennans*, *C. luteo-olivacea* and *C. malorum* have be published (Köhl et al., 2013, 2018). The approximately 2000 stored DNA samples from the two earlier studies were used for quantification of DNA of *Stemphylium* spp. and pear-pathogenic *S. vesicarium.*

Fungal isolates and DNA extraction

For the validation of a genus-specific Taqman PCR assay for quantification of DNA of *Stemphylium* spp. in environmental samples (see below), isolates of *Stemphylium* spp. originating from various hosts or necrotic tissues, including isolates obtained from pear, grass, asparagus and onion in an earlier study (Köhl et al., 2009a), isolates of *Alternaria* spp. (Table 1) and all 72 non-target fungal isolates listed in Köhl et al. (2018), representing 28 fungal species belonging to 18 genera, were collected (Table 1 and Table 1 in Köhl et al., 2018).

Alternaria spp. and *Stemphylium* spp. isolates were grown for two weeks at 18 °C on potato dextrose agar (PDA). Mycelium and spores of cultured isolates were scraped from the agar surface, freeze-dried and macerated by bead-beating for 10 s at 5000 beats per minute (bpm) in a ribolyser (Hybaid). Subsequently, DNA was extracted using the DNeasy plant mini kit (Qiagen, Westburg, Germany) with the following modification. The prescribed incubation at 65 °C in lysis buffer was prolonged from 10 min to 1 h. The DNA was eluted in two times 50 µl and kept at −20 °C for further use. DNA of the other fungal isolates were obtained as described in Köhl et al. (2018).

Development of Taqman PCR assay for *Stemphylium* spp.

An alignment was made of the ITS sequences of all *Stemphylium* spp. isolates published by Woudenberg et al. (2017) with the software CLC genomics workbench 12.0 (CLC bio). Primer express v. 3.0.1 (Thermo Fisher Scientific) was used for the in silico design of primers and probe for ITS regions that are genus-specific for *Stemphylium* spp. The specificity of the developed Taqman PCR assay was tested using 1 ng DNA of all *Stemphylium* spp. isolates (Table 1) and of all non-target fungi (Table 1 and Table 1 in Köhl et al., 2018). The test was considered specific if, for the non-target fungi, no reactions or C_t values were measured that were higher than the C_t value for the target fungus at the lowest concentration of the dynamic range of the TaqMan PCR assay. The sensitivity of the assay was determined for isolate

Table 1 Fungal isolates used in the study and their origin

Fungal species	Isolate number	Host	Location	Year
Alternaria alternata	CBS 154.31	American bladdernut	USA	Unknown
A. alternata	424	Cauliflower	Groessen, Netherlands	2007
A. alternata	03011	Potato	Zeewolde, Netherlands	2003
Alternaria brassicae	164	Unknown	Netherlands	Unknown
Alternaria brassicicola	177	Cauliflower	Netherlands	1991
Alternaria solani	04008	Potato	Marknesse, Netherlands	2004
Stemphylium amaranthi	17041	Barnyard grass	Netherlands	2017
Stemphylium beticola	17128	Sugar beet	Netherlands	2017
Stemphylium botryosum	315 [b]	Pear	Limburg, Netherland	2003
S. botryosum	CBS 714.68	Burclover	Canada	1954
Stemphylium eturmiunum	17028	Sugar beet	Netherlands	2017
Stemphylium lycopersici	CBS 321.87	Tomato	Senegal	Unknown
Stemphylium solani	CBS 408.54	Tomato	USA	1952
Stemphylium vesicarium	266 [a]	Asparagus	Montanaso, Italy	1992
S. vesicarium	301 [b]	Pear	Binderveld, Belgium	2003
S. vesicarium	849 [c]	Pear	Spain	Unknown
S. vesicarium	850	Pear fruit	Leerbroek, Netherlands	2002
S. vesicarium	852	Pear fruit	Krabbendijke, Netherlands	2002
S. vesicarium	859	Necrotic grass leaf	Randwijk, Netherlands	2003
S. vesicarium	861	Necrotic grass leaf	Rossum, Netherlands	2003
S. vesicarium	865	Necrotic pear leaf	Rossum, Netherlands	2003
S. vesicarium	868	Necrotic pear leaf	Wijk bij Duurstede, Netherland	2003
S. vesicarium	877	Necrotic grass leaf	Randwijk, Netherlands	2003
S. vesicarium	886	Necrotic pear leaf	Randwijk, Netherlands	2003
S. vesicarium	891	Necrotic pear leaf	Velddriel, Netherlands	2003
S. vesicarium	893	Necrotic pear leaf	Rossum,Netherlands	2003
S. vesicarium	898	Onion leaf	Rhenen, Netherlands	2003
S. vesicarium	900	Onion leaf	Marknesse, Netherlands	2003
S. vesicarium	903	Onion leaf	Westmaas, Netherlands	2003
S. vesicarium	911	Pear fruit	Vianen, Netherlands	2003
S. vesicarium	915	Pear fruit	's Gravenpolder, Netherlands	2003
S. vesicarium	918	Pear fruit	Lexmond, Netherlands	2003
S. vesicarium	920	Pear fruit	Numansdorp, Netherlands	2003
S. vesicarium	927	Onion leaf	Nagele, Netherlands	2003
S. vesicarium	933	Pear fruit	Dinteloord, Netherlands	2003
S. vesicarium	938	Asparagus leaf	Rinkesfort, Netherlands	2004
S. vesicarium	946	Asparagus leaf	Roggel, Netherlands	2004
S. vesicarium	955	Asparagus leaf	Netherlands	2004
S. vesicarium	958	Asparagus leaf	Netherlands	2004
S. vesicarium	17022	Barnyard grass	Netherlands	2017
S. vesicarium	17060	Orache	Netherlands	2017
S. vesicarium	17064	Wheat	Netherlands	2017
S. vesicarium	17072	Knotweed	Netherlands	2017
S. vesicarium	17079	Maize	Netherlands	2017
S. vesicarium	17085	Potato	Netherlands	2017
S. vesicarium	17123	Sugar beet	Netherlands	2017

Table 1 (continued)

Fungal species	Isolate number	Host	Location	Year
S. vesicarium	17179	Sugar beet	Netherlands	2017
S. vesicarium	493B/93 [a]	Pear fruit	Viallaparo, Italy	1993
S. vesicarium	88/6 [a]	Pear fruit	Baricella, Italy	1994
S. vesicarium	BR11 [a]	Pear fruit	Altedo, Italy	1994
S. vesicarium	EPS2 [c]	Pear fruit	Girona, Spain	1991
S. vesicarium	W94	Apple leaf	Randwijk, Netherlands	2002
S. vesicarium	CBS 311.92	Necrotic onion leaf	Netherlands	1991

Isolates are from the collection of Wageningen University and Research unless indicated otherwise.

[a] Instituto di Entomologia e Patologia vegetale, Università Cattolica S. Cuore, Piacenza, Italy.

[b] Kindly provided by P. Creemers, pcfruit, Gorsem, Belgium.

[c] Kindly provided by I. Llorente, Institute of Food and Agricultural Technology, University of Girona, Girona, Spain.

The fungal culture strains starting with CBS were obtained from the Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands.

S. vesicarium 493B/93, using five replicate dilution series prepared in nuclease free water in steps of 10^{-1} starting with 1000 pg down to 0.01 pg.

Protocol of Taqman PCR assays

Three separate Taqman PCR assays were performed in a 384-well format in CFX 384 (Bio-Rad) Real-Time PCR Detection System to quantify (1) DNA of *Stemphylium* spp., (2) DNA of pear-pathogenic *S. vesicarium* (Köhl et al., 2009b) and (3) DNA encoding green fluorescent protein (GFP) serving as an amplification control (AC) (Klerks et al., 2004). For each TaqMan PCR assay, 1 μL sample was mixed with 9 μL reaction mix containing 5 μL 2X PerfeCTa Toughmix no ROX (Quantabio); and, for *Stemphylium* spp., 200 nM Minor Groove Binder (MGB) probe and 300 nM of the forward primer and 300 nm of the reverse primer; for pear-pathogenic *S. vesicarium*, 200 nM probe (FAM-CTTGACATAGGCT GGT AAATGGGTCT-3IABkFQ) and 320 nM of each forward primer (TCTCATCGGAGGCAGTGATG) and reverse primer (TGGAGATATCACGGAAAA TGAGTTC); and for GFP 120 nM probe (FAM-AAC CATTACCTGTCCACACAATCTGCCC-3IABkFQ) and 320 nM of each forward primer (TGGCCCTGT CCTTTTACCAG) and reverse primer (TTTTCGTTG GGATCTTTCGAA) and 0.8 pg GFP DNA. The reaction conditions for each Taqman PCR were: 95 °C for 2 min; 40 cycles of 95 °C for 15 s followed by 60 °C for 60 s.

A tenfold serial dilution ranging from 1 ng to 10 fg of genomic DNA of *S. vesicarium* isolate 493B/93 was included in each 384-well plate for reference. If measurements of the AC amplification indicated inhibition of TaqMan PCRs, measurements were repeated after 2- and tenfold dilution of the sample. The concentrations of extracted pathogen DNA in the samples were calculated from the derivative cycle threshold values (C_t values) of TaqMan PCR assays for the DNA dilution series and for DNA extracts of plant samples, and expressed as pg DNA of *Stemphylium* spp. per mg plant residue (dry weight) and as pg DNA of pear-pathogenic *S. vesicarium* per mg plant residue (dry weight) (SvPP). Additionally, for each sample the DNA concentration of *Stemphylium* spp. non-pathogenic in pear (SNPP) was estimated by subtraction of the measured DNA concentration of SvPP from the measured concentration of DNA of *Stemphylium* spp.

Results

Genus-specific *Stemphylium* qPCR assay

Specificity A genus-specific *Stemphylium* qPCR assay was developed based on the Minor Groove Binder (MGB) probe FAM-CACCCATGTCTT TTG-BHQ1 (Applied Biosystems), forward primer GGGCTCCAGCTTGTCTGAA and reverse primer CGCCCAGGAAACAAGAAGTG. Primers were

synthesized by IDT (Integrated DNA Technologies). A blastn search of the primer–probe combination gave no match with the whole genome of *Pyrus* (biosample PRJNA381668) and *Malus domestica* (biosample SAMN02981243). C_t values between 17.1 and 19.5 were measured for 1 ng genomic DNA of 47 *Stemphylium* spp. isolates from different hosts, locations and years listed in Table 1. DNA of the wide range of other fungi and yeasts, pathogenic to pear or commonly present in orchards as saprophytes, did not amplify, confirming the highly specific amplification of the newly developed qPCR assay.

Sensitivity The standard curve obtained with *S. vesicarium* 493B/93 showed a linear dynamic range from 1000 pg to 0.01 pg corresponding to a C_t range of 17—36 (Fig. 1). Linear regression for the $\log_{10}$-transformed amount of S. *vesicarium* 493B/93 DNA (ng) and the corresponding C_t values revealed r^2 of 0.995. The limit of detection was determined at 10 fg DNA per reaction. PCR inhibition was observed when 0.8 pg of gfp DNA was added to the samples collected from 2009–2011 containing *S. vesicarium*

in plant matrix. After a two-fold dilution of these samples no inhibition was observed (data not shown). The samples collected in 2012, showed no inhibition.

Dynamic of *Stemphylium* spp. in two pear orchards 2009–2011

A total of 735 samples collected in two pear orchards cv. Conference in Erichem and Hansweert in 2009–2011 has been analyzed. Samples of dead pear leaves, fallen pear fruits, pear prunings, dead grass leaves, and dead weeds. *Stemphylium* spp. were detected in 723 samples whereas in only 12 samples no *Stemphylium* spp. were detected. The majority of these negative samples were obtained from prunings. The average concentration of *Stemphylium* spp. in positive samples was 255 pg DNA mg^{-1} plant tissue (dw). The maximum concentration was found in a sample of dead weeds at 17,050 pg DNA mg^{-1} plant tissue (dw). Pear-pathogenic *S. vesicarium* was detected in 188 samples (26% of samples) with an average concentration of positive samples at 58 pg DNA mg^{-1} plant tissue (dw). The maximum
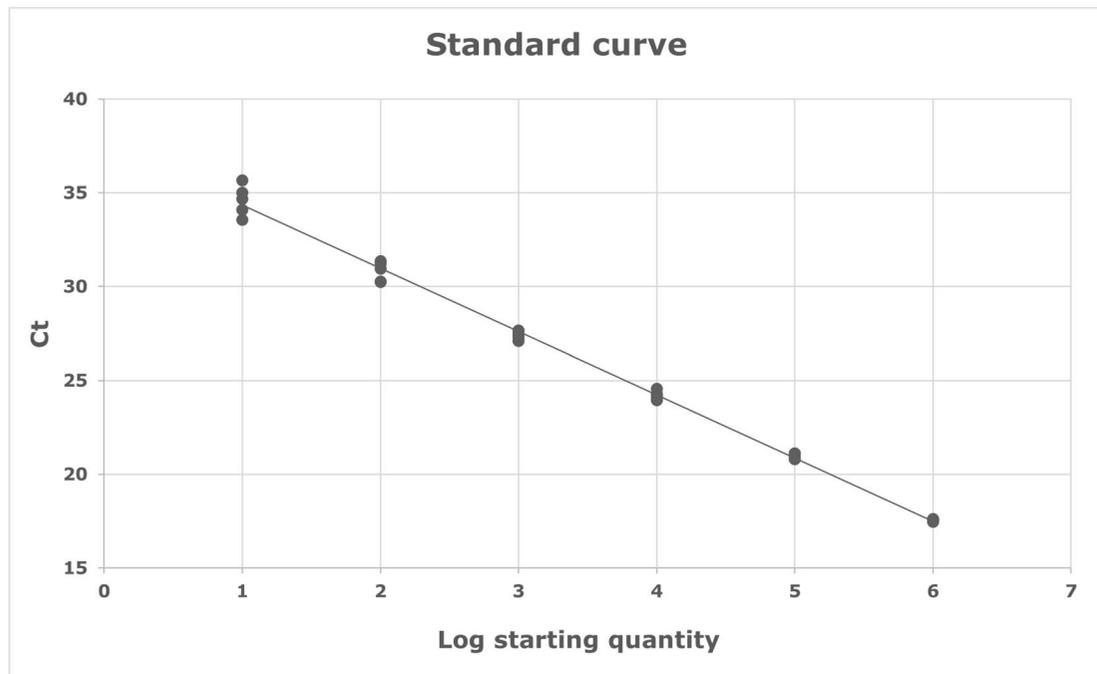

Fig. 1 Standard curve of *S. vesicarium* genomic DNA $\log_{10}$ concentration against the cycle threshold (Ct) values. The concentration range was 1000 pg to 0.01 pg DNA per reaction, each amount tested in five-fold. Linear regression equation of the standard curve was: $Y = -3.37X + 37.721$ at $R^2 = 0.995$

concentration, found in a sample of dead pear leaves, was 988 pg DNA mg^{-1} plant tissue (dw). In 35 cases, subtraction of DNA concentration of pear-pathogenic *S. vesicarium* from the DNA concentration of *Stemphylium* spp. resulted in negative values. In a part of these cases, both measured concentrations were close to the detection limit and negative values can be explained by variation in qPCR measurements, especially at higher Ct value levels. However, in 16 cases differences were above 10 pg DNA mg^{-1} plant tissue (dw). Values for non-pathogenic *Stemphylium* spp. were corrected for these cases and replaced by zero to avoid negative values. *Stemphylium* spp. non-pathogenic to pear (SNPP) was detected in 689 samples (94% of the samples) with an average concentration of 253 pg DNA mg^{-1} plant tissue (dw) for positive samples. The maximum concentration (found in dead weed tissue) was 16,877 pg DNA mg^{-1} plant tissue (dw).

The dynamics of *Stemphylium* spp. populations non-pathogenic in pear (SNPP) and of pear-pathogenic *S. vesicarium* (SvPP) in dead pear leaves, fallen pear fruits, pear prunings, dead grass leaves, and dead weeds during the period from February 2009 to May 2011 are shown in Fig. 1 using ln-transformed values. Back transformed values are shown in Supplementary Fig. 1. Values for SvPP fluctuated during the seasons in the sampled substrate types, with generally higher values during the late growing season and the winter season and with lower values during the early growing seasons (Fig. 2). SNPP DNA concentrations were higher, often for several magnitudes, than concentrations of SvPP in nearly all cases (Fig. 2; Supplementary Fig. 1). Only in fallen leaves from the orchard in Erichem in 2009, the SNPP:SvPP ratio was lower, with higher values for SvPP than for SNPP in few cases (Fig. 2A; Supplementary Fig. 1A). In substrate types with SvPP present, such as fallen leaves in Hansweert in 2009, fluctuations for SvPP and SNPP showed similar pattern, for examples highest concentrations for both groups were observed in September 2009 (Fig. 2B). It was not observed that an increase of one of the two *Stemphylium* measurements is coincided by a decrease of the other measurement. Pronounced differences between the two orchards were not only observed for SvPP but also for SNPP. In the orchard in Hansweert both groups generally reached much higher concentrations compared to Erichem. This striking differences in DNA concentrations for

SNPP between the two orchards was found for tissues from pears, leaves, fruits and prunings (Fig. 2A-F), as well as for plant residues from weeds and grasses, which are no hosts for SvPP (Fig. 2G-J). There is a trend that concentrations in all such substrate types were higher in Hansweert during the 2009 season and were closer to the values found in Erichem in the 2010 season.

The amount of fallen pear leaves per m^2 had been estimated in the orchards in Erichem and Hansweert at all sampling dates (Köhl et al., 2013). This allows an estimation of the dynamics of the amounts of DNA of SvPP and SNPP in fallen pear leaves on the orchard floors (Fig. 3). There are consistent peaks of SNPP late in the growing season and during winter with clearly higher peak in Hansweert compared to Erichem. The observed peaks for SNPP coincided with peaks of SvPP that reached much lower levels (Fig. 3; Supplementary Fig. 2).

Stemphylium spp. colonization of various substrates in 10 pear orchards in August 2009

Stemphylium spp. were detected in 94 samples of a total of 95 samples. The average concentration of *Stemphylium* spp. in positive samples was 891 pg DNA mg^{-1} plant tissue (dw). The maximum concentration was found in a sample of dead weeds at 20,092 pg DNA mg^{-1} plant tissue (dw). Pear-pathogenic *S. vesicarium* (SvPP) was detected in 50 samples (53%) with an average concentration of positive sample at 50 pg DNA mg^{-1} plant tissue (dw). The maximum concentration, found in a samples of a dead weed (*Senecio vulgaris*), was 1,352 pg DNA mg^{-1} plant tissue (dw). In three cases, subtraction of DNA concentration of pear-pathogenic *S. vesicarium* from the DNA concentration of *Stremphylium* spp. in negative values. Values for non-pathogenic *Stemphylium* spp. for these cases were replaced by zero. *Stemphylium* spp. non-pathogenic to pear (SNPP) was detected in 86 samples (91%) with an average concentration of 919 pg DNA mg^{-1} plant tissue (dw) for positive samples. The maximum concentration, found in dead weed tissue (*Senecio vulgaris*), was 20,092 pg DNA mg^{-1} plant tissue (dw).

Data from the various substrates collected in August 2009 in 10 pear orchards confirmed that SNPP is almost ubiquitously present in the various substrates at the different locations (Fig. 4;
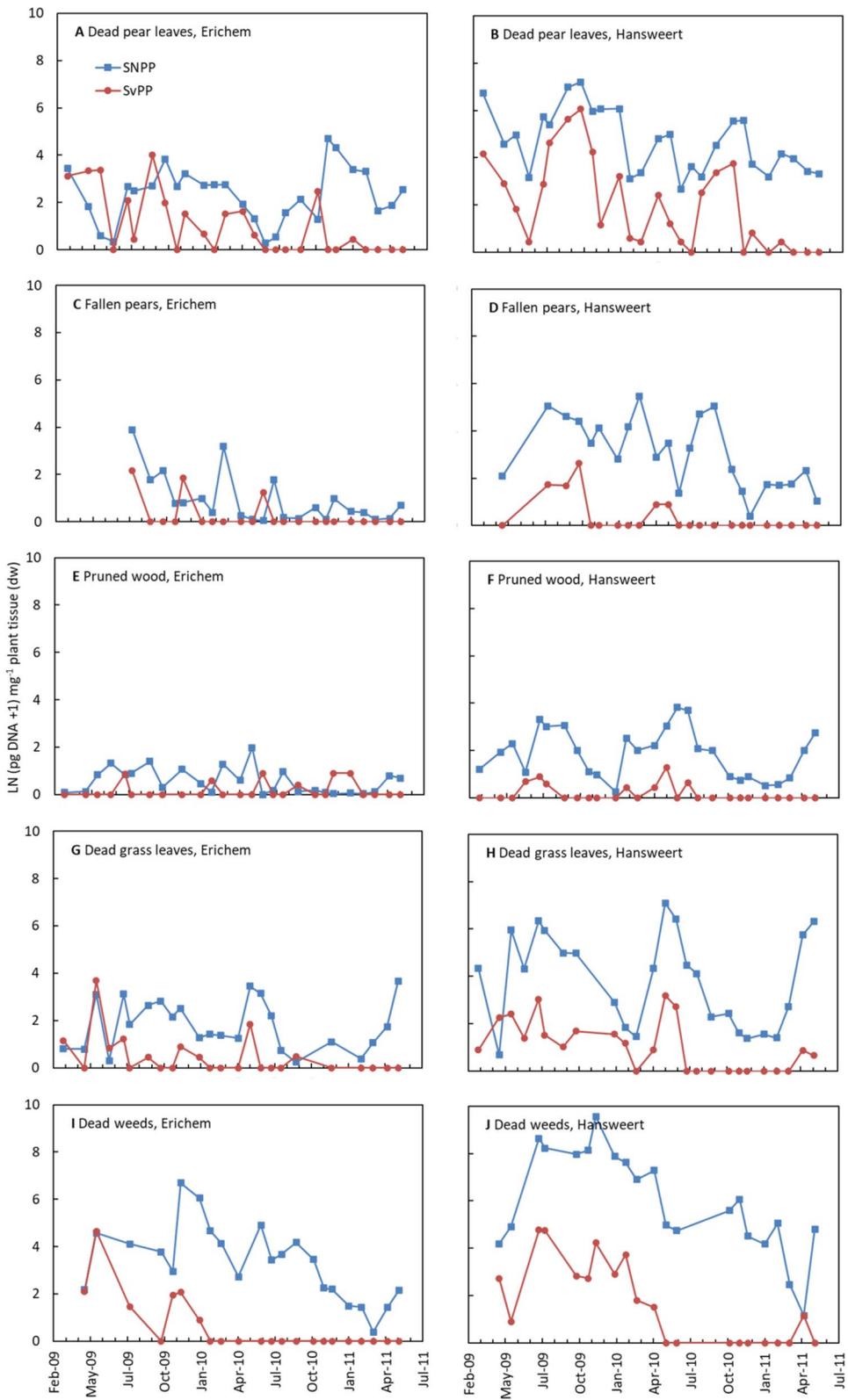
◄**Fig. 2** Dynamics of *Stemphylium* spp. populations of pear-pathogenic S. *vesicarium* (SvPP) and non-pathogenic in pear (SNPP) on (**A**, **B**) dead pear leaves, (**C**, **D**) fallen pear fruits, (**E**, **F**) pruned wood, (**G**, **H**) dead grass leaves, and (**I**, **J**) dead weeds in two pear orchards cv. Conference in (**A**, **C**, **E**, **G**, **I**) Erichem and (**B**, **D**, **F**, **H**, **J**) Hansweert. LN-transformed concentration of *Stemphylium* DNA (ln pg DNA mg.$^{-1}$ of plant tissue (dry weight))

Supplementary Fig. 3). Highest concentration were found in dead pear leaves and dead weeds whereas pruned wood contained the lowest concentrations of *Stemphylium* spp. non-pathogenic to pear (SNPP). Pear-pathogenic *S. vesicarium* (SvPP) showed highest concentrations in fallen pear leaves, dead weeds and in two samples of fallen pear fruits. Only in fours samples, SvPP was found without presence of SNPP. In the majority of all other cases, SNPP reached higher concentrations compared to SvPP. In dead weeds, the variation between samples and orchards was highest for both SvPP and SNPP. There is a common trend that high concentrations of SNPP co-incidence with high concentrations of SvPP.

Stemphylium spp. colonization of various substrates collected in ten pear orchards and ten apple orchards in May 2021

DNA of *Stemphylium* spp. and of pear-pathogenic *S. vesicarium* was quantified in a total of 529 samples of necrotic plant tissues and the top soil layer in the samples originated from ten apple orchards and ten pear orchards. Substrate types sampled in May 2021 in four replicates per orchard were: cankers, leaf litter of apple or pear, mummies, prunings, fruit spurs, dead weeds, dead grass, champost (if applied in the orchards), and soil (for details see: Köhl et al., 2018). DNA of *Stemphylium* spp. was found in 467 samples (88%) with a mean of 21 pg DNA mg^{-1} plant tissue for positive samples (Table 2). The maximum concentration measured was 4053 pg DNA mg^{-1} plant tissue in an apple mummy. DNA of pear-pathogenic *S. vesicarium* was detected in seven samples. The mean concentration for such positive samples was 0.8 pg SvPP DNA mg^{-1} plant tissue, the maximum concentration was 4.7 pg SvPP DNA mg^{-1} plant tissue in a dead pear leaf. Since pear-pathogenic *S. vesicarium* was almost negligible, values calculated for non-pear-pathogenic *Stemphylium* spp. (SNPP) was close to the measured concentrations of DNA of *Stemphylium* spp was 466 positive samples

with a mean of 22 pg SNPP DNA mg^{-1} plant tissue for positive samples and a maximum of 4053 pg pg SNPP DNA mg^{-1} plant tissue.

There was a considerable variation in colonization of plant tissues by *Stemphylium* spp. between orchards (Fig. 5 and 6). For example, dead apple leaves from apple orchards 5, 6 and 7 showed high colonization of all sampled apple tissues and of residues of weeds and gras, whereas much lower concentrations were found in the other apple orchards.
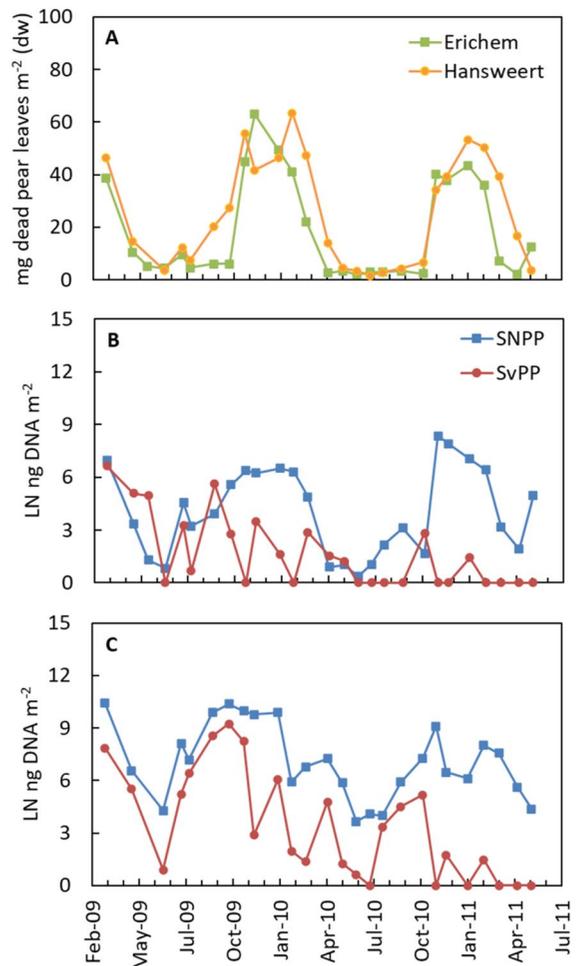

Fig. 3 Dynamics of the amounts of DNA of pear-pathogenic *S. vesicarium* (SvPP) and *Stemphylium* spp. non-pathogenic to pear (SNPP) in fallen pear leaves on the orchard floors. (**A**) Amount of dead pear leaves on orchard floors (modified from Köhl et al., 2013), (**B**) Amount of *Stemphylium* DNA m^{-2} orchard floor in Erichem (LN ng DNA m^{-2}), (**C**) Amount of *Stemphylium* DNA m^{-2} orchard floor in Hansweert (LN ng DNA m^{-2})

Fig. 4 Concentration of pear-pathogenic S. *vesicarium* (SvPP) and *Stemphylium* spp. non-pathogenic in pear (SNPP) in (**A**) dead pear leaves, (**B**) fallen pear fruits, (**C**) pruned wood, (**D**) dead grass leaves, and (**E**) dead weeds in samples collected in 10 pear orchards cv Conference in August 2009. LN pg DNA mg^{-1} (dry weight)
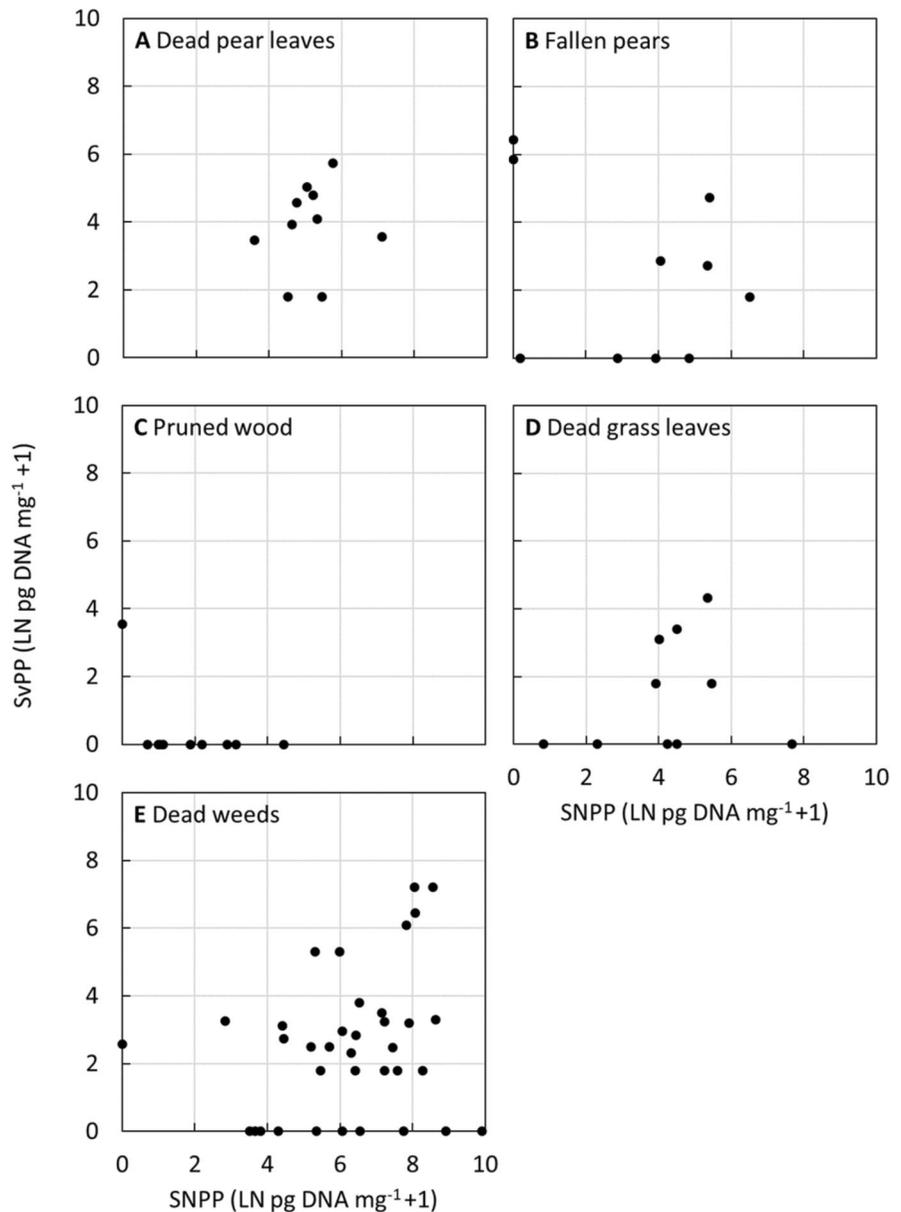
A similar variation was found between pear orchards with, for example, highest values in pear orchard 17 in residues of weeds and grasses and consistently low values in pear orchard 12.

Dynamics of *Stemphylium* spp. in various substrates in four pear orchards and four apple orchards in May to December 2012

The dynamics in *Stemphylium* spp. colonization was followed using samples of leaf litter of apple or pear,

mummies, pruned wood, dead weeds, dead grass collected in May (see above) and additionally from June to December 2012 in four apple orchards and four pear orchards (for details see: Köhl et al., 2018). DNA of *Stemphylium* spp. and of pear-pathogenic *S. vesicarium* was quantified in a total of 671 samples of necrotic plant tissues collected in June to December. DNA of *Stemphylium* spp. was found in 655 samples (98%) with a mean of 51 pg DNA mg^{-1} plant tissue for positive samples. The maximum concentration measured was 932 pg DNA mg^{-1} plant tissue in

a dead weed collected in an pear orchard in August. DNA of pear-pathogenic *S. vesicarium* was detected in ten samples. The mean concentration for such positive samples was 3 pg SvPP DNA mg^{-1} plant tissue, the maximum concentration was 14 pg SvPP DNA mg^{-1} plant tissue in a dead pear leaf collected in September. Since pear-pathogenic *S. vesicarium* was almost negligible, values calculated for non-pear-pathogenic *Stemphylium* spp. (SNPP) was close to the measured concentrations of DNA of *Stemphylium* spp. *Stemphylium* spp. was present in all orchards in fallen leaves of pear and apple, mummies of apple and residues of weeds and grasses during the whole period from May until December (Fig. 7). There are indications, that *Stemphylium* spp. colonization differs between orchards, e.g. highest values were found for apple orchard 7 and values for apple orchard 3 tended to be lower.

Discussion

Brown spot caused by *S. vesicarium* occurred in Dutch pear orchards in the late 1970s in the Po Valley in Italy and has been reported in the Netherlands and Belgium since the early 1990 s (Polfliet, 2002; Ponti et al., 1982). Since that, Dutch growers experience brown spot as a high risk potentially causing severe losses through fruit rot. Losses due to brown spot vary between seasons. A particular observation is that brown spot appears seasonally in a fraction of Dutch pear orchards, often coinciding with high losses. Risk factors for such affected orchards are still not known. As a consequence of such unpredictable risks, growers are using fungicides during the season to secure pears from potential brown spot losses, although such schedules are needed only in a limited number of orchards where brown spot develops

Table 2 DNA concentration of non-pear-pathogenic *Stemphylium* spp. (SNPP) and pear-pathogenic *Stemphylium vesicarium* (SvPP) in different substrates collected in ten apple orchards and ten pear orchards in May 2012

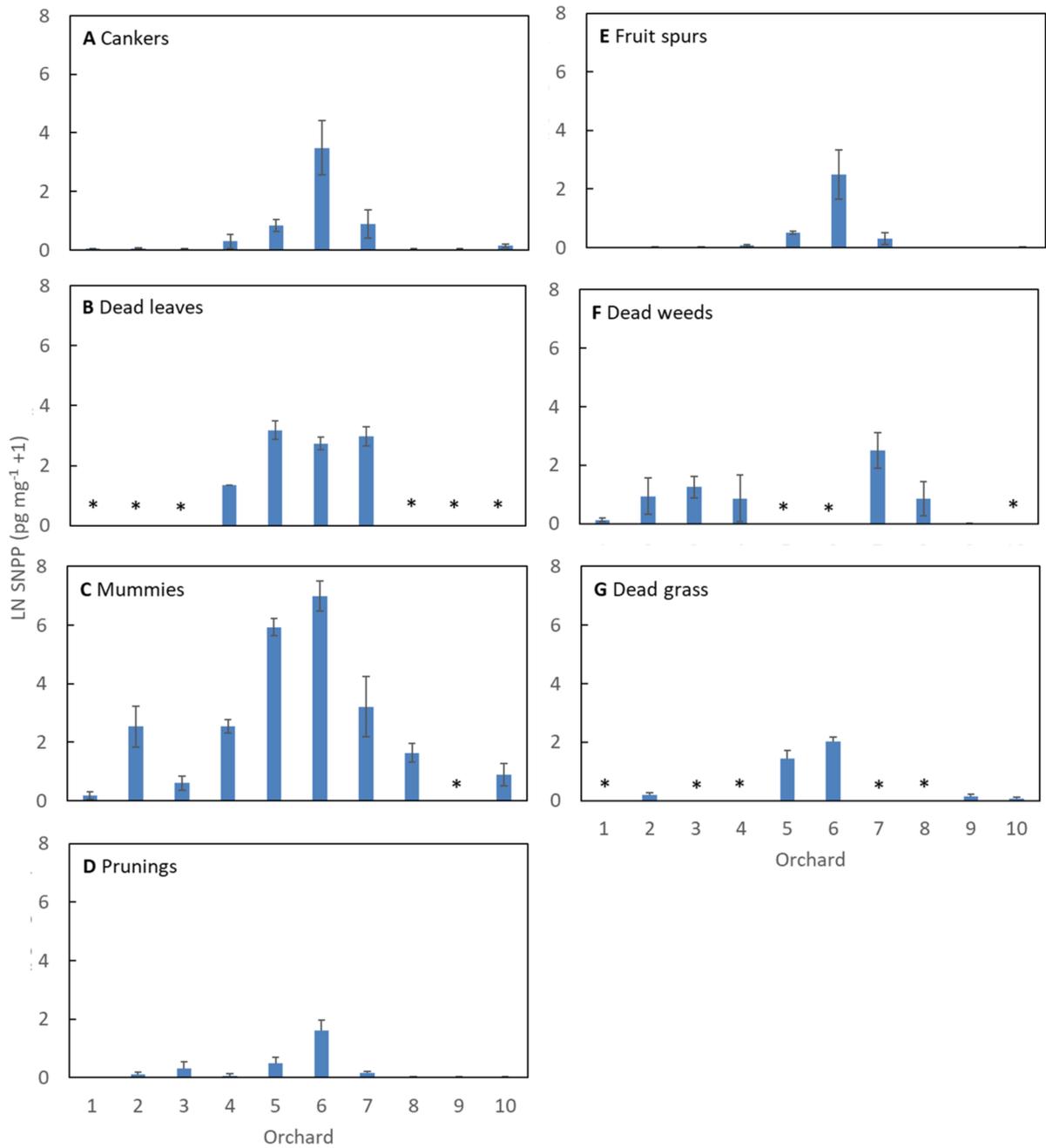| Substrate	Total number of samples	No. of samples with SNPP	pg SNPP DNA mg^{-1} plant tissue (dry weight)		No. of samples with SvPP	pg SvPP DNA mg^{-1} plant tissue (dry weight)	
			Average of positive samples	Maximum		Average of positive samples	Maximum
Apple							
Cankers	40	40	8	167	0	-	-
Apple leaf litter	13	13	20	51	0	-	-
Mummies	36	36	237	4053	0	-	-
Prunings	40	39	0.7	10	0	-	-
Fruit spurs	40	40	4	149	0	-	-
Dead weeds	27	27	6	63	0	-	-
Dead grass	20	20	2	10	0	-	-
Champost	8	8	0.1	0.5	0	-	-
Soil	40	16	0	0.2	0	-	-
Total	264	239			0		
Pear							
Cankers	40	40	0.1	2	0	-	-
Pear leaf litter	36	35	6	34	6	1	5
Mummies	6	6	0	0	0	-	-
Prunings	40	36	0.1	4	0	-	-
Fruit spurs	40	33	0	0.1	0	-	-
Dead weeds	32	32	6	97	0	-	-
Dead grass	19	19	10	116	1	<0.1	<0.1
Champost	12	12	0.1	0.4	0	-	-
Soil	40	14	0	0.1	0	-	-
Total	265	227			7		

Fig. 5 DNA concentration of non-pear-pathogenic *Stemphylium* spp. (SNPP) in (**A**) cankers, (**B**) dead apple leaves, (**C**) mummies, (**D**) prunings, (**E**) fruit spurs, (**F**) dead weeds, and (**G**) dead grass collected in ten apple orchards in May 2012. Means and standard errors of the mean for four replicates. '*' indicate orchards where plant tissue was not available

in a growing season but are obsolete in many other orchards. Understanding factors resulting in high brown spot damage in particular orchards in a particular season will thus reduce the use of preventative spray schedules in orchards, save the environment and reduce costs for growers.

It is known that haplotype variants within *S. vesicarium* exist that are either pear-pathogenic or
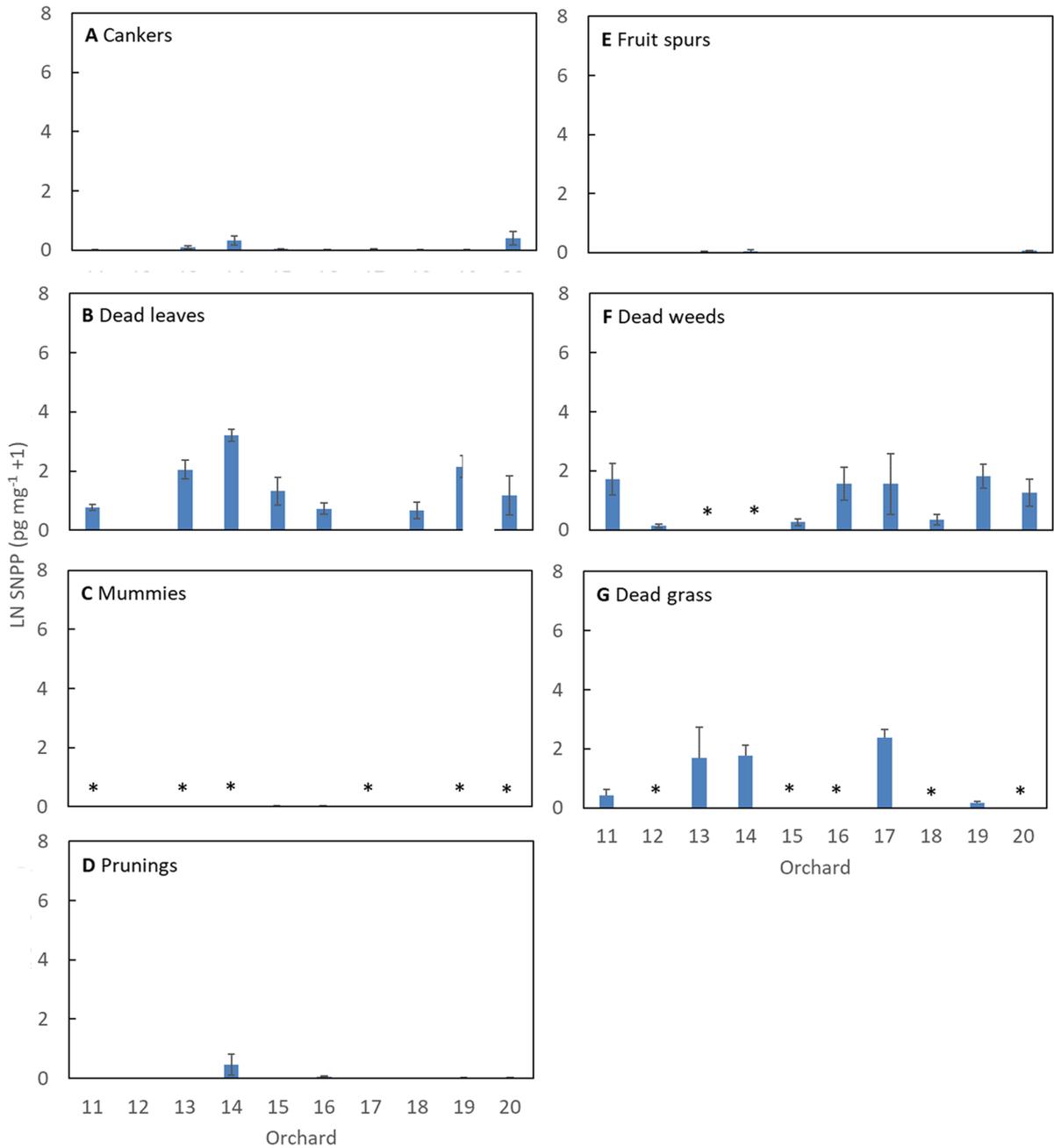
Fig. 6 DNA concentration of non-pear-pathogenic *Stemphylium* spp. (SNPP) in (**A**) cankers, (**B**) dead pear leaves, (**C**) mummies, (**D**) prunings, (**E**) fruit spurs, (**F**) dead weeds, and (**G**) dead grass collected in ten pear orchards in May 2012. Means and standard errors of the mean for four replicates. '*' indicate orchards where plant tissue was not available

non-pathogenic in pear (Köhl et al., 2009a). Pear-pathogenic haplotypes are host-specific due to the production of host-specific toxins (Singh et al., 1999) and have the saprophytic potential to colonize

infected host tissue of pear as well as necrotic tissues of pear and non-hosts. Pear-pathogenic strains have been frequently isolated from various necrotic tissues in pear orchards. Their seasonal population
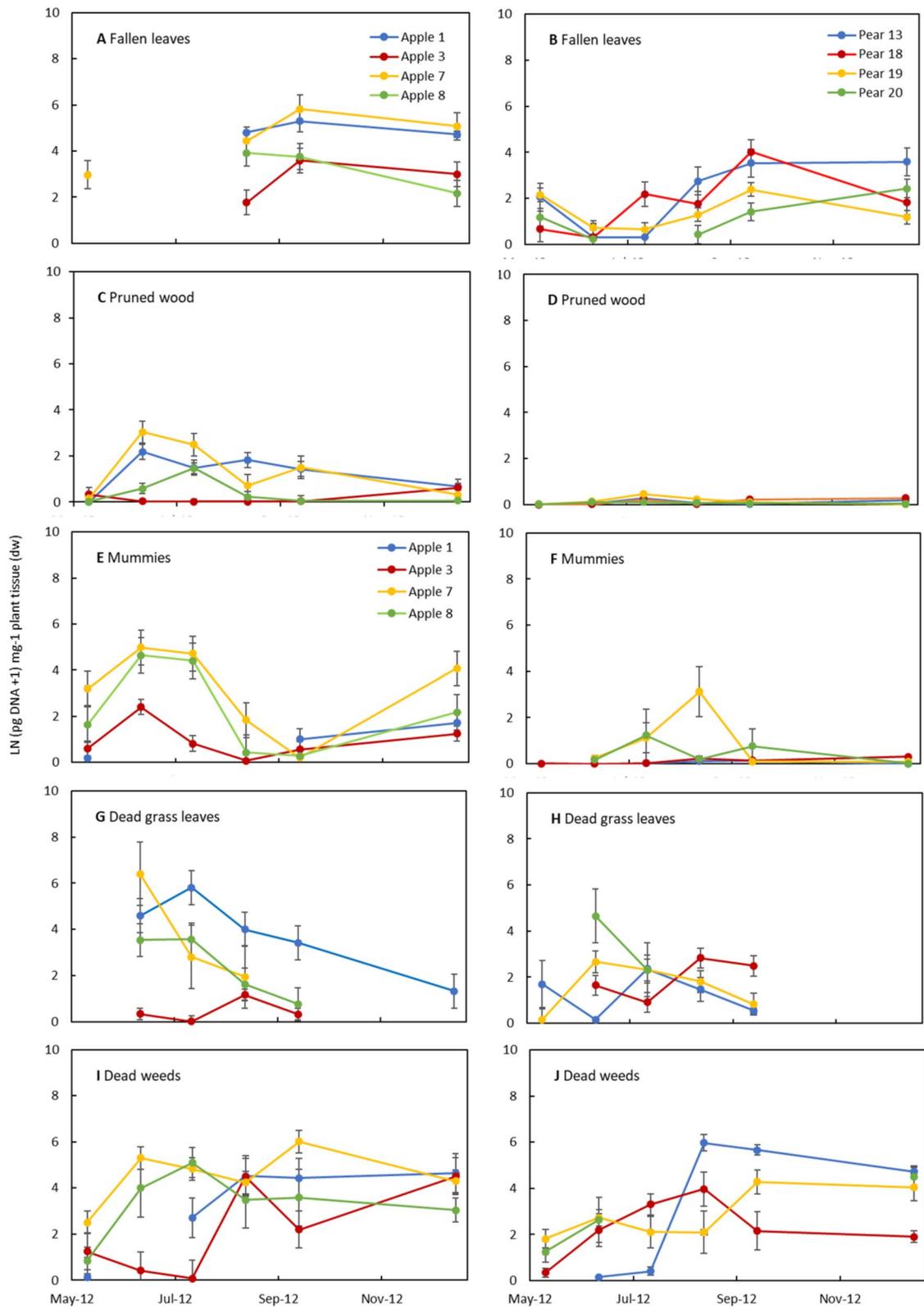
◄ **Fig. 7** Dynamics of concentration of non-pear-pathogenic *Stemphylium* spp. in (**A**, **B**) fallen leaves, (**C**, **D**) pruned wood), (**E**, **F**) mummies, (**G**, **H**) dead grass leaves, and (**I**, **J**) dead weeds collected in (**A**, **C**, **E**, **G**, **I**) four apple orchards and (**B**, **D**, **F**, **H**, **J**) four pear orchards in May – December 2012. Bars represent standard errors of mean of four replicates per sampling date

dynamics in such tissues have been described using qPCR specific for the pear-pathogenic *S. vesicarium* haplotype (Köhl et al., 2009b, 2013). Necrotic tissues on orchard floors including dead weeds and grasses have been identified as main sources of *S. vesicarium* ascospore and conidia causing infections on pear leaves and fruits during the season (Moragrega et al., 2018; Rossi et al., 2008).

The genus *Stemphylium* has been described as ubiquitous saprophytic colonizers of dead plant tissues (Hudson, 1971). Saprophytic haplotypes of *S. vesicarium* have been isolated from pear tissues and non-host tissues in pear orchards (Köhl et al., 2009a) and have also been recovered by spore trapping in the air within pear orchards (Moragrga et al., 2018). We hypothesized that there is competition for substrate and space between saprophytic populations of *Stemphylium* spp. and populations of pear-pathogenic *S. vesicarium.* Saprophytic *Stemphylium* spp. populations may reach sizes close to the carrying capacity of available necrotic plant tissues present in an orchard (Chapman & Byron, 2018). In this situation, pear-pathogenic *S. vesicarium* will not be sustainably supported in this environmental niche. Such a competition may contribute to resilience of orchards against brown spot attacks if saprophytic populations would suppress pear-pathogenic populations. It also would allow new options to enhance saprophytic populations e.g. by enhancing *Stemphylium* by inoculation of specific hosts grown on the orchard floor to multiply *Stemphylium* populations being pathogenic in such hosts but not in pear. If this was the case, careful use of fungicides applied to control brown spot should be considered to avoid interference with beneficial saprophytic *Stemphylium* populations.

Two large sets of samples of necrotic tissues from orchards from earlier research were explored to obtain first insights for the validation of the hypothesis. DNA concentrations of *Stemphylium* spp. and of pear-pathogenic *S. vesicarium* were quantified in samples collected in pear orchards in 2009–2011 that had been selected specifically for research on pear brown spot. The other set of evaluated samples

had been originally collected in apple and pear orchards for research on fruit rot diseases different from brown spot. The obtained results clearly showed that *Stemphylium* spp. are ubiquitously present in orchards colonizing necrotic tissues originating from the grown crops or other plants. Highest concentrations of *Stemphylium* spp. were found in tissue from apple and pear leaves, weeds, and grasses but low concentrations were found on prunings. There is a trend that apple tissues support higher *Stemphylium* spp. concentrations compared to pear tissues. A remarkable variation of *Stemphylium* spp. population size was measured between different orchards. Pear-pathogenic *S. vesicarium* was found in samples collected in 2012 in pear orchards specifically selected for research on brown spot epidemiology, as reported earlier (Köhl et al., 2013). However, in the pear orchards randomly selected regarding brown spot in 2012 and in the additionally monitored apple orchards, pear-pathogenic *S. vesicarium* was almost absent. Only in few cases low amounts were measured. These findings contribute to the general observations in the Netherlands that brown spot is not a ubiquitous disease but occurs occasionally in distinct pear orchards.

Co-occurrence of both pear-pathogenic *S. vesicarium* and *Stemphylium* spp. regarded as non-pathogenic to pear were found in approximately 25% of the samples from pear orchards collected 2009–2011. Concentrations of *Stemphylium* spp. DNA were consistently higher than concentrations of pear-pathogenic *S. vesicarium*. Concentrations of pear-pathogenic *S. vesicarium* tended to be much lower than concentrations of other *Stemphylium* spp. These results indicate that substrate conditions in combination with environmental conditions favored the development of both populations. This pattern has been found for samples collected in various orchards in August 2009 (Fig. 4) but is even more visible for samples collected in two orchards during a period from February 2009 to May 2011 (Fig. 2 and 3). Fluctuations for both *Stemphylium* spp. and pear-pathogenic *S. vesicarium* show the same pattern, e.g. with peaks in fallen pear leaves in October 2009 and October 2010. This was found for the pear orchard at Hansweert at substantially higher colonization levels compared to the pear orchard at Erichem. No cases were found were increased concentrations of *Stemphylium* spp. coincided with a decrease in

concentrations of pear-pathogenic *S. vesicarium*. The hypothesis that high colonization by saprophytic *Stemphylium* spp. will outcompete pear-pathogenic populations can thus not be supported.

The exploration of DNA concentrations of *Stemphylium* spp. and pear-pathogenic *S. vesicarium* in the approximately 2000 samples broadly collected in pear and apple orchards in different years lead to the conclusion that it is not very likely that populations of *Stemphylium* spp., naturally occurring in the orchards or enhanced in population size through inundation as biological control agents, may outcompete pathogenic *S. vesicarium* populations in necrotic tissues of pear and other plants present on orchard floors. However, substrate competition can still be a powerful function of microbiomes present in the plant residues in orchards increasing the resilience against brown spot or for the development of biological control agents (Kerdraon et al., 2019; Köhl & Fokkema, 1998). Experiments with *Trichoderma* spp. showed promising results (Llorente et al., 2012; Moragrega et al., 2021). Future research is needed for the screening of a new generation of saprophytic antagonists with strong competitive saprophytic abilities and niche occupation abilities.

Data Availability The datasets generated during the study are available on reasonable request.

Declarations

Ethical approval This article does not contain any studies with human participants and animal performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare no conflict of interest and that the research complies with ethical standards. This research does not involve any human participants and/or animals.

References

Aveling, T. A. S., & Snyman, H. G. (1993). Infection studies of *Stemphylium vesicarium* on onion leaves. *Mycological Research, 97*, 984–988. https://doi.org/10.1016/S0953-7562(09)80866-6

Bani, A., Pioli, S., Ventura, M., Panzacchi, P., Borruso, L., Tognetti, R., Tonon, G., & Brusetti, L. (2018). The role of microbial community in the decomposition of leaf litter and deadwood. *Applied Soil Ecology, 126*, 75–84. https://doi.org/10.1016/j.apsoil.2018.02.017

Berg, G., Kusstatscher, P., Abdelfattah, A., Cernava, T., & Smalla, K. (2021). Microbiome modulation—toward a better understanding of plant microbiome response to microbial inoculants. *Frontiers in Microbiology, 12*,. https://doi.org/doi.org/10.3389/fmicb.2021.650610

Cavina, F., Baiocco, S., Tomba, L., Ravaglia, F., Moretti, C., Raso, R., Rettore, V. G., Parrilli, M., Pradolesi, G., & Bugiani, R. (2024). A New Source of Inoculum for *Stemphylium vesicarium*: Consequences for the Management of Brown Spot of Pear. *Agronomy, 14*, 2522. https://doi.org/10.3390/agronomy14112522

Chaisrisook, C., Stuteville, D. L., & Skinner, D. Z. (1995). Five *Stemphylium* spp. pathogenic to alfalfa: Occurrence in the United States and time requirements for ascospore production. *Plant Disease, 79*, 369–372.

Chapman, E. J., & Byron, C. J. (2018). The flexible application of carrying capacity in ecology. *Global Ecology and Conservation, 13*,. https://doi.org/10.1016/j.gecco.2017.e00365

Cong, Z., Xinlong, P., Kong, B. H., Yueqiu, H., Jun, M., Yongcui, M., & Keqiang, C. (2022). First report of *Stemphylium eturmiunum* causing leaf spot and black spot on apple in Zhaotong, Yunnan, China. *Plant Disease*, *106*, doi.org/https://doi.org/10.1094/PDIS-08-21-1834-PDN

Cowling, W. A., Gilchrist, D. G., & Graham, J. H. (1981). Biotypes of *Stemphylium botryosum* on alfalfa in North America. *Phytopathology, 71*, 679–684.

De Jong, P. F., & Heijne, B. (2008). Exclusion of the inoculum source of brown spot (*Stemphylium vesicarium*. *Acta HOrticulturae, 800*, 833–838. https://doi.org/10.17660/ActaHortic.2008.800.113

Falloon, P. G., Falloon, L. M., & Grogan, R. G. (1987). Etiology and epidemiology of Stemphylium leaf spot and purple spot of asparagus in California. *Phytopathology, 77*, 407–413.

Hanse, B., Raaijmakers, E. E. M., Schoone, A. H. L., & van Oorschot, P. M. S. (2015). *Stemphylium* sp., the cause of yellow leaf spot disease in sugar beet (*Beta vulgaris* L.) in the Netherlands. *European Journal of Plant Pathology, 141*, 1–12. https://doi.org/10.1007/s10658-015-0617-8

Hausbeck, M. K., Hartwell, J., & Byrne, J. M. (1999). Epidemiology of Stemphylium leaf spot and purple spot in no-till Asparagus. *Acta Horticulturae, 479*, 205–210. https://doi.org/10.17660/ActaHortic.1999.479.28

Hudson, H. J. (1971). The development of the saprophytic fungal flora as leaves senesce and fall. Pages 447–455 in: Ecology of Leaf Surface Micro-Organisms. T. F. Preece and C. H. Dickinson, eds. Academic Press, London, New York.

Kerdraon, L., Laval, V., & Suffert, F. (2019). Microbiomes and pathogen survival in crop residues, an ecotone between plant and soil. *Phytobiomes J., 3*, 246–255. https://doi.org/10.1094/PBIOMES-02-19-0010-RVW

Klerks, M. M., Zijlstra, C., & van Bruggen, A. H. C. (2004). Comparison of realtime PCR methods for detection of *Salmonella enterica* and *Escherichia coli* O157: H7, and introduction of a general internal amplification control. *Journal of Microbiological Methods, 59*, 337–349. https://doi.org/10.1016/j.mimet.2004.07.011

Köhl, J., Groenenboom-de Haas, B. H., & Wenneker, W. (2023). *Fibulorhizoctonia psychrophila*, causal agent of lenticel spot in pome fruit, rarely detected in Dutch pear and apple orchards. *European Journal of Plant Pathology, 166*, 285–289. https://doi.org/10.1007/s10658-023-02663-6

Köhl, J., & Fokkema, N. J. (1998). Biological control of necrotrophic foliar fungal pathogens. In G. J. Boland & L. V. Kuykendall (Eds.), *Plant-Microbe Interactions and Biological control* (pp. 49–88). Marcel Dekker.

Köhl, J., de Jong, P.-F., Kastelein, P., Groenenboom-de Haas, B. H., Anbergen, R. H. N., Balkhoven, H., & Wubben, J. P. (2013). Dynamics of pear-pathogenic *Stemphylium vesicarium* in plant residues in Dutch pear orchards. *European Journal of Plant Pathology, 137*, 609–619. https://doi.org/10.1007/s10658-013-0274-8

Köhl, J., Groenenboom-de Haas, B. H., Goossen-van de Geijn, H. M., Speksnijder, A., Kastelein, P., de Hoog, S., & Gerrits van der Ende, B. (2009). Pathogenicity of *Stemphylium vesicarium* from different hosts causing brown spot in pear. *European Journal of Plant Pathology, 124*, 151–162. https://doi.org/10.1007/s10658-008-9402-2

Köhl, J., Groenenboom-de Haas, B. H., Kastelein, P., Rossi, V., & Waalwijk, C. (2009). Quantitative detection of pear-pathogenic *Stemphylium vesicarium* in orchards. *Phytopathology, 99*, 1377–1386. https://doi.org/10.1094/PHYTO-99-12-1377

Köhl, J., Wenneker, M., Groenenboom-de Haas, B. H., Anbergen, R., Goossen-van de Geijn, H. M., Lombaers-van der Plas, C. H., Pinto, F. A. M. F., & Kastelein, P. (2018). Dynamics of post-harvest pathogens *Neofabraea* spp. and *Cadophora* spp. in plant residues in Dutch apple and pear orchards. *Plant Pathology, 67*, 1264–1277. https://doi.org/10.1111/ppa.12854

Koike, S. T., Henderson, D. M., & Butler, E. E. (2001). Leaf spot disease of spinach in California caused by *Stemphylium botryosum*. *Plant Disease, 85*, 126–130. https://doi.org/10.1094/PDIS.2001.85.2.126

Leuprecht, B. (1990). *Stemphylium botryosum* Wallr. on asparagus. *Gesunde Pflanzen, 42*, 187–191.

Llorente, I., & Montesinos, E. (2006). Brown spot of pear: An emerging disease of economic importance in Europe. *Plant Disease, 90*, 1368–1375. https://doi.org/10.1094/PD-90-1368

Llorente, I., Moragrega, C., Ruz, L., & Montesinos, E. (2012). An update on control of brown spot of pear. *Trees, 26*, 239–245. https://doi.org/10.1007/s00468-011-0607-1

Llorente, I., Vilardell, A., Vilardell, P., Pattori, E., Bugiani, R., Rossi, V., & Montesinos, E. (2010). Control of brown spot of pear by reducing the overwintering inoculum through sanitation. *European Journal of Plant Pathology, 128*, 127–141. https://doi.org/10.1007/s10658-010-9637-6

Moragrega, C., Puig, M., Ruz, L., Montesinos, E., & Llorente, I. (2018). Epidemiological features and trends of brown spot of pear disease based on the diversity of pathogen populations and climate change effects. *Phytopathology, 108*, 223–233. https://doi.org/10.1094/PHYTO-03-17-0079-R

Moragrega, C., Carmona, A., & Llorente, I. (2021). Biocontrol of *Stemphylium vesicarium* and *Pleospora allii* on pear by *Bacillus subtilis* and *Trichoderma* spp.: Preventative and curative effects on inoculum production. *Agronomy, 11*, 1455. https://doi.org/10.3390/agronomy11081455

Polfliet, M. (2002). Aantasting Stemphylium neemt met het jaar toe [Infection of Stemphylium increases every year]. *Fruitteelt, 92*(20), 16–17.

Ponti, I., Cavanni, P., & Brunelli, A. (1982). Maculatura bruna delle pere: Eziologia e difesa. *Informatore Fitopatologico, 32*, 35–40.

Prados-Ligero, A. M., González-Andújar, J. L., Melero-Vara, J. M., & Basallote-Ureba, M. J. (1998). Development of *Pleospora allii* on garlic debris infected by *Stemphylium vesicarium*. *European Journal of Plant Protection, 104*, 861–870.

Prencipe, S., & Spadaro, D. (2021). First report of *Stemphylium eturmiunum* causing postharvest rot on tomato (*Solanum lycopersicum*) in Italy. *Plant Disease, 105*, 3756. https://doi.org/10.1094/PDIS-11-20-2389-PDN

Rossi, V., Pattori, E., & Bugiani, R. (2008). Sources and seasonal dynamics of inoculum for brown spot disease of pear. *European Journal of Plant Pathology, 121*, 147–159. https://doi.org/10.1007/s10658-007-9258-x

Schneider, T., Keiblinger, K. M., Schmid, E., Sterflinger-Gleixner, K., Ellersdorfer, G., Roschitzki, B., Richter, A., Eberl, L., Zechmeister-Boltenstern, S., & Riedel, K. (2012). Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. *ISME Journal, 6*, 1749–1762. https://doi.org/10.1038/ismej.2012.11

Spadoni, A., Ippolito, A., & Sanzani, S. M. (2020). First report of *Stemphylium eturmiunum* causing postharvest rot of sweet cherry in Italy. *Crop Protection, 132*,. https://doi.org/10.1016/j.cropro.2020.105112

Voriskova, J., & Baldrian, P. (2013). Fungal community on decomposing leaf litter undergoes rapid successional changes. *ISME Journal, 7*, 477–486. https://doi.org/10.1038/ismej.2012.116

Woudenberg, J. H. C., Hanse, B., van Leeuwen, G. C. M., Groenewald, J. Z., & Crous, P. W. (2017). *Stemphylium* revisited. *Studies in Mycology, 87*, 77–103. https://doi.org/10.1016/j.simyco.2017.06.001