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Infection conditions for *Neofabraea perennans* and *Phacidiopycnis washingtonensis* on developing apple fruit in the orchard

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Abstract In Northern Germany, a major share of postharvest losses of apple fruit is due to preharvest infections by pathogenic fungi. Little is known about their infection biology. Inoculation experiments were conducted with the most important storage-rot pathogen *Neofabraea perennans*, as well as with the recently discovered minor rot *Phacidiopycnis washingtonensis*, by spraying developing fruit on apple trees with conidial suspensions to drip wetness between June and harvest time (September / October). All inoculation events in three trial seasons were chosen to coincide with natural rainfall. Phenological stages and meteorological parameters of each infection event were used for correlation analyses. Both pathogens produced increasing fruit rot levels with inoculation dates closer to harvest. In addition, for *N. perennans* seven environmental factors were positively correlated with disease incidence, the most significant ones being the duration of post-infection leaf wetness and the scab infection quotient incorporating wetness and temperature. With *P. washingtonensis*, in addition to fruit maturity three environmental factors were identified. In a second step, multifactorial models for both pathogens were created using the phenological and meteorological factors. For *N. perennans*, scab infection quotient until first drying-off, dry hours within the leaf wetness period and post-inoculation precipitation levels were identified as important factors, whereas for *P. washingtonensis* only the average temperature during the leaf wetness period had a significant influence on the rot incidence. Either model was extended by the viability of conidia used for inoculation. Possibilities to deploy these models for a more accurate a priori prediction of the likely severity of storage rot and a more targeted use of pre- and postharvest fungicides and physical postharvest treatments are discussed.

Keywords Bull's eye rot · Postharvest · Rubbery rot · Speck rot · Storage rot

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Apple (*M. domestica*) is the major crop in northern Europe's largest fruit-growing area, the Lower Elbe region of Northern Germany, where it is produced on >10,000 ha under conditions of integrated pest management (IPM; approx. 85% of the total acreage) or organic production (15%). In 2017, the most important cultivars in IPM were Elstar and the Jonagold group comprising 33.4% and 28.4% of the total acreage, respectively, as well as Braeburn with 11.5%, Boskoop with 5.9% and Holsteiner Cox with 4.7% (Görgens 2017). Under the maritime regional climate, fungal postharvest diseases are a major source of crop losses in short-term cold storage in ambient air, as well as in long-term storage (> 3 months) of fruit under ultra-low oxygen (ULO) or dynamically controlled atmosphere (DCA) conditions.

In the Lower Elbe region as well as other parts of northern Europe, commercial losses to storage rots often amount to 2–10% in IPM, and 10–30% in organic production, although much higher losses may occur in particular batches of fruit or in particular years (Maxin et al. 2014). For example, in 1968 up to 90% of all harvested fruit were lost to storage rots (Blank 1971).

A wide diversity of storage-rot fungi may be found on Northern German apples, and their species composition may vary over time. Until 1968 *Neofabraea perennans* (syn. *Pezicula perennans*, anam. *Cryptosporiopsis perennans*, '*Gloeosporium perennans*'; see Chen et al., 2016) and *Phlyctema vagabunda* (syn. *Pe. alba, N. alba*, *N. vagabunda*, '*G. album*'; see Chen et al., 2016) were the major storage-rot fungi, the former species being dominant. Both species were effectively controlled by methyl benzimidazole carbamate (MBC) fungicides from 1969 until the mid-1980s when resistance to these fungicides began to build up (Weber and Palm 2010). During that period, the apple canker fungus *Neonectria ditissima* (syn. *N. galligena*) became the dominant storage-rot species. During the past 25 years, *N. perennans* and *P. vagabunda* have again contributed about 65–80% of all losses to storage rots, other important species being *Botrytis cinerea*, *N. ditissima*, *Monilinia fructigena* and *Penicillium expansum* (Schulte 1997; Weber 2009). Rubbery rot due to *Phacidiopycnis washingtonensis*, known from North America since 2003 (Xiao et al. 2005), was discovered in the Lower Elbe region in 2009 as the first record from Europe (Weber 2011). It has become established as an omnipresent but minor rot, contributing about 5% of all rots in long-term storage, occasionally more (Maxin et al. 2014). The above order of importance of storage-rot fungi seems to be similar in many other European countries, the exception being that *P. vagabunda* usually dominates over *N. perennans* in regions to the west (Wenneker et al. 2016), south-west (Giraud and Bompeix 2012), south-east (Michalecka et al. 2016; Pešicová et al. 2017), and south (Kennel 1988) of Northern Germany. In Scandinavia (Rasmussen and Jepsen 1958; Talvia 1960; Olsson 1965; Tahir et al. 2009; Weber 2009; Tahir 2019) and north-eastern Europe (Borecki 1961; Michalecka et al. 2016), however, *N. perennans* is of greater importance but the proportion depends on cultivar (Kaspers 1967) and locality (Palm and Kruse 2005). Further, *Colletotrichum acutatum*, which is rare in Northern Germany, is often a major cause of storage rot in the western part of Scandinavia (Børve et al. 2013; Børve and Stensvand 2017; Tahir 2019).

With the exception of *P. expansum*, all storage-rot fungi seem to infect apples before harvest, causing a delayed outbreak of symptoms with advancing fruit maturity in long-term storage (Maxin et al. 2014). Because of this long latency stage, postharvest treatments of fruit with fungicides (Palm and Kruse 2012a; Holthusen 2014; Aguilar et al. 2018) or with hot water dipping or rinsing (Maxin et al. 2012) are feasible. Although these two types of postharvest treatment are being practised to a modest extent in IPM and organic production, respectively, preharvest fungicide sprays remain essential in IPM for controlling storage rots as well as storage scab caused by infections of *Venturia inaequalis*. Unfortunately, even repeated sprays with captan, followed by single sprays with trifloxystrobin, fludioxonil, pyrimethanil or cyprodinil, give only limited efficacies of approx. 50–75% (Palm and Kruse 2012b). One reason for this may lie in our limited understanding of the infection biology of the relevant pathogens.

Several storage-rot fungi have been described from other regions to cause infections of apple fruit throughout the vegetation period, but increasingly so during a 4- to 6-week period before harvest (Edney 1958; Borecki 1961; Spotts 1985; Henriquez et al. 2008; Kim et al. 2014; Sikdar et al. 2014; Aguilar et al. 2017). However, no such data are available for Northern Germany. Further, because most previous studies included artificial elements such as inoculating detached fruit (Edney 1956; Díaz et al. 2019), immersing fruit in conidial suspensions (Olsson 1965), or wrapping inoculated fruit in bags (Edney 1958; Kim and Xiao 2006; Henriquez et al. 2008; Sikdar et al. 2014; Aguilar et al. 2017; Díaz et al. 2019), we still lack important details of infection conditions, such as duration of surface wetness, the role of brief dry periods, and temperature. In addition, there are no European data for the recently discovered *P. washingtonensis*. Therefore, we conducted a series of trials in which fruit-bearing apple trees were spray-inoculated with spore suspensions of *N. perennans* and *P. washingtonensis* under natural conditions, and harvested fruit were scored for the incidence of rots during prolonged storage periods. Assuming moisture to be a key factor, we targeted the fruit inoculations to natural precipitation events, attempting to characterise the effects of moisture in relation to other parameters a posteriori.

Materials and methods

Location and inoculation

Orchards belonging to the Esteburg Fruit Research and Advisory Centre (Jork, Northern Germany; 53.506° N, 9.751° E) were used for all experiments. These orchards were managed according to standard IPM practices except that in experimental years no fungicides were applied between end of blossom and harvest. Trees were grafted on M9 rootstock and were cultivated as slender spindles (approx. 3 m height) with a planting distance of 1 m within rows and 3.5 m between rows. All orchards were in full production (6–13 y old). Inoculation experiments were conducted with cultivars Braeburn during the 2016 and 2018 seasons, and Pinova in 2017 and 2018. For each time-point and fungal species, four individual trees with uniform and high fruit set were randomly selected for inoculation. A buffer of at least one tree was left between two inoculated trees.

Weather data were collected using a UNIKLIMA vario weather station (TOSS GmbH, Potsdam, Germany) equipped with a combi sensor for temperature (LT1) and relative air humidity (RLF), two leaf wetness-dew sensors (BLN1), and a heated pluviometer with a 0.1 mm double tipping scale. The weather station (altitude 0 m) was located on the Esteburg site about 900 m away from the orchards used for our experiments.

For inoculation of apple trees, spores were harvested in water from cold-stored fruit infected in the previous season, filtered through sterile cotton wool, adjusted to 5×10^5 conidia ml^{-1}, and kept in cold-room storage at 2 °C for a maximum of 6 h prior to use. Both fungi were identified by lesion morphology and by the size and shape of their conidia as compared to those of reference isolates identified by ITS sequence analysis (Weber 2011; Maxin et al. 2014). On each chosen tree, all fruit were inoculated to drip wetness with 300 ml spore suspension, corresponding to 32.9 ± 15.0 µl (16,450 ± 7520 conidia) on the first inoculation date and 65.53 ± 27.38 µl (32,765 ± 13,690 conidia) just before harvest. Inoculation was conducted directly after a rain, during a rain gap or within 60 min before the onset of an expected rainfall, thereby ensuring prolonged leaf wetness after inoculation. The fruit size ranged from 35 to 45 mm diam. on the first inoculation date, corresponding to the phenological stages BBCH 74–75 (Meier 2001), to final fruit size just before harvest. At each time-point, the remaining spore suspension was used to check spore viability by incubating 100 µl suspension for 24 h at room temp. on potato dextrose agar augmented with 200 mg penicillin G and 200 mg streptomycin sulphate (all reagents supplied by Carl Roth GmbH + Co. KG, Karlsruhe, Germany). The percentage of germination among 100 randomly selected spores on the agar surface was determined by microscopy using an Axio Scope.A1 and a ×40 objective (Carl Zeiss, Göttingen, Germany).

Storage and evaluation

Following inoculation with *N. perennans* or *P. washingtonensis*, four trees of each cultivar and inoculation time-point were completely harvested at commercially relevant dates for long-term storage. Four uninoculated control trees from the same orchards with a buffer of at least one tree to inoculated trees were also harvested. All fruit from individual trees were stored together in 1–2 wooden boxes (20 kg capacity) in a cold-room at 2 °C in ambient atmosphere and were examined after approx. 3, 6 and 8 months post-harvest. At each of these times, apples showing symptoms of storage rot were separated and further stored in display trays at 2 °C until the causal fungus could be identified by microscopic examination of conidia produced on disease lesions. For each tree, primary data were summarised as the number of fruits infected with either *N. perennans* or *P. washingtonensis* as well as the total number of fruits.

Statistical analysis

Primary data were arcsine square root transformed and statistically analysed using the software R (version 4.0.3; R Core Team 2020) accessed by the RStudio user surface (version 1.3.1073; RStudio Team 2020). An ANOVA was conducted on the data, followed by computation of the mean separation using the LSD method ($P < 0.05$) to reveal if the disease incidence in the inoculated trees was higher than in the untreated control trees. The LSD method ($P < 0.05$) was also used to determine whether the incidence of disease between 'Braeburn' and 'Pinova' differed in pairwise comparisons of different inoculation dates in the 2018 trials.

As the first statistical approach, linear regression analyses were performed for *N. perennans* or *P. washingtonensis* individually to reveal the impact of inoculation dates on mean disease incidence. This was

followed by linear regression analyses performed on disease incidences of individual trees of all experimental years to reveal the impact of meteorological and phenological parameters on either *N. perennans* or *P. washingtonensis* infections. Linear regressions were only kept if statistically significant ($P < 0.05$). As a second step, generalised linear models were computed using the meteorological and phenological parameters. Model complexity was reduced by iterative removal of factors with the least impact. *F* tests were conducted to reveal statistical differences ($P < 0.05$) between the original and the simplified model. Simplified models were only kept if no statistical differences occurred.

Results

In each season of our trials, 6–8 inoculation dates at intervals of 14–21 d were realised, beginning about 6–9 weeks after the end of flowering and ending one or a few days before harvest. The ranges and average numbers of fruit harvested from each tree were as follows: 49–215 fruit (av. 119.8 fruit) on 'Braeburn' 2016; 57–258 fruit (av. 159.2 fruit) on 'Pinova' 2017; 90–329 fruit (av. 168.0 fruit) on 'Braeburn' 2018; and 46–220 fruit (av. 127.8 fruit) on 'Pinova' 2018. The incidence of disease was evaluated as the cumulative share of infected fruit at the end of 8 months of cold-room storage. Attempts to score the severity of disease (number of lesions per fruit) were unsuccessful because the lesions became visible in the course of several months, meaning that early lesions had already covered large proportions of fruit surface by the time when late lesions became visible.

The results showed a general trend of increasing fruit infestation during the weeks before harvest on cvs Braeburn and Pinova both for *N. perennans* (Fig. 1) and for *P. washingtonensis* (Fig. 2). In several cases this trend was statistically significant on the basis of the raw data, as shown. The germination rates of spores for both species were generally at or above 90%, except for 23 Sept. 2018 when, for reasons unknown to us, only 20% (*N. perennans*) or 10% (*P. washingtonensis*) of the spores germinated in our test. By eliminating 25 Aug. 2018 from the analyses due to the low mean temperature (9.9 °C) and the very short leaf wetness period (12 h), which resulted in a scab infection quotient <90% (Mills and Laplante 1954), and 23 Sept. 2018 due to the low spore germination rate, stronger trends for increasing fruit infestation during the weeks before harvest resulted for *N. perennans* both on 'Braeburn' 2018 ($R^2 = 0.59$, $P = 0.073$) and on 'Pinova' 2018 ($R^2 = 0.69$, $P = 0.081$), whereas these trends became significant for *P. washingtonensis* on 'Braeburn' 2018 ($R^2 = 0.84$, $P = 0.01$) and 'Pinova' 2018 ($R^2 = 0.88$, $P = 0.018$) (data not shown).

Infestation levels following artificial inoculation with either species were almost always significantly ($P < 0.05$) higher than in the uninoculated control even on the first inoculation dates. However, inoculation with *P. washingtonensis* failed to produce high infestation levels on 23 Sept. 2018, when poor spore germination was the likely explanation, and on 23 June 2018 ('Pinova' only) as well as on 25 Aug. 2018 ('Braeburn' only). Higher infestation levels were consistently obtained with *N. perennans* than *P. washingtonensis* despite an identical inoculum load.

An analysis of cultivar-specific responses was possible only for the 2018 season. For both pathogens, a comparison of all data including uninoculated controls showed no significant difference between 'Braeburn' and 'Pinova'. Further, for *N. perennans* pairwise comparisons between the last, penultimate and third-last inoculation dates showed a significantly elevated infection in 'Pinova' only for the penultimate date, i.e. 'Pinova' on 6 Sept. versus 'Braeburn' on 23 Sept. ($P < 0.05$), whereas for *P. washingtonensis* significantly elevated infection levels were observed for 'Pinova' versus 'Braeburn' on 6 Sept. versus 2 Oct. ($P < 0.05$) and 13 Aug. versus 6 Sept. ($P < 0.05$).

Whilst our efforts to time inoculation to impending rainfall were successful, the rain events varied in their amount of precipitation and in the length of surface wetness, thereby offering (i) post-inoculation precipitation (mm) and (ii) leaf wetness (h) as potential variables for statistical analysis. Leaf wetness was defined as a period of at least 10% wetness as indicated by one or both leaf-wetness-dew sensors, interrupted by a maximum of 6 h continuous dry time. Other variables were (iii) the apple scab infection quotient (scqu in %; Mills and Laplante 1954) until the end of a leaf wetness period; (iv) apple scab infection quotient until the end of a scab infection period after 10–16 h continuous drought according to the definition in Northern Germany (Klopp 2020); (v) temperature (°C) as well as (vi) degree-hours (°h) during a leaf wetness period; (vii) periods of early surface dryness during a leaf wetness period; (viii) periods of surface dryness after the end of a leaf wetness period but still within a scab infection

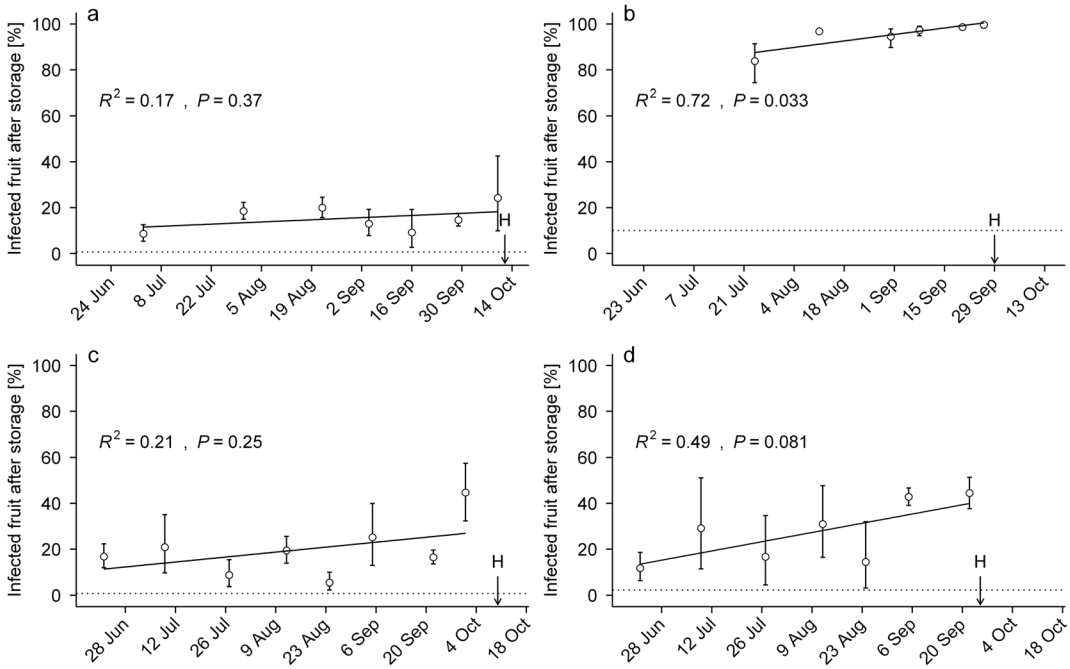

Fig. 1 Mean (±SE) incidence of postharvest fruit rot due to *Neofabraea perennans* following artificial inoculation of fruit in the orchard at various dates before harvest, in comparison to the uninoculated control (dotted line). The trials were (a) cv. Braeburn in 2016, (b) cv. Pinova in 2017, (c) cv. Braeburn in 2018, and (d) cv. Pinova in 2018. Arrows indicate harvest dates (H)

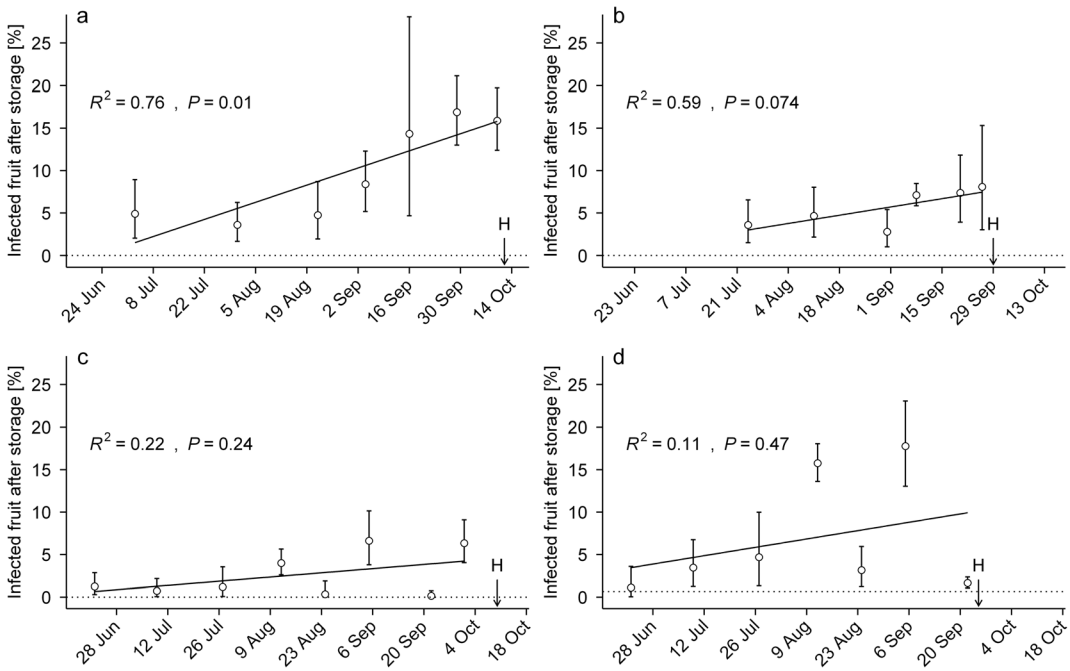

Fig. 2 Mean (±SE) incidence of postharvest fruit rot due to *Phacidiopycnis washingtonensis* following artificial inoculation of fruit in the orchard at various dates before harvest, in comparison to the uninoculated control (dotted line). The trials were (a) cv. Braeburn in 2016, (b) cv. Pinova in 2017, (c) cv. Braeburn in 2018, and (d) cv. Pinova in 2018. Arrows indicate harvest dates (H)

period (h); (ix) onset of rain after inoculation (h); and (x) inoculation date (d before harvest). In total, for each pathogen 28 inoculation time-points were available for analysis. Eight variables were found to have a significant impact ($P < 0.05$) on the infestation level with *N. perennans* (Fig. 3a-h), while only four variables were found to have a significant impact ($P < 0.05$) on the infestation level with *P. washingtonensis* (Fig. 4a-d). The influencing variables differed considerably between the two pathogens. The scab infection quotient and the post-inoculation leaf wetness period had the largest impact on the *N. perennans* infestation level. In contrast, inoculation date as well as the number of surface dryness hours after a leaf wetness period but still within a scab infection period were most important for the *P. washingtonensis* infection level.

The exceptionally high infection levels for *N. perennans* in the 2017 season (Fig. 1b) were obtained in an unusually wet season, giving 173.3 mm cumulative rainfall during July to September, as compared to 61.1 mm in the unusually dry 2018 season. No elevated infections were observed for *P. washingtonensis* (Fig. 2b). Since high values for almost all of the wetness-related data points were obtained for *N. perennans* in 2017, that year has a strong influence on data analysis. By excluding the 2017 data for *N. perennans*, significant ($P < 0.05$) impacts were determined for days before harvest and post-inoculation precipitation (not shown).

Variables were also used to create generalised linear models which could explain the infestation levels even better than a single variable. Because the germination rate of spores was low on 23 Sept. 2018, 'germinable spores' was introduced as an additional variable. Logistic regression revealed that the logit of the *N. perennans* incidence was a function of inoculation date (d before harvest, DBH), scab infection quotient during a leaf wetness period, surface dryness hours within a leaf wetness period, post-inoculation precipitation, and the concentration of germinable spores:

$$\ln\left(\frac{p}{1-p}\right) = -3.291 + 0.036*DBH + 0.019*scqu_{during\ a\ leaf\ wetness\ period}\ (\%) + 0.492*surface\ dryness\ hours_{during\ a\ leaf\ wetness\ period} - 0.283*mm\ precipitation_{post-inoc.} + 0.245*\frac{spores_{germinable}}{10^5} ml^{-1}$$

The standard errors of the six parameters providing the elements of the function (−3.291, zero; 0.036, DBH; 0.019, scqu during a leaf wetness period; 0.492, surface dryness hours; −0.283, precipitation; and 0.245, spores) were 0.581, 0.004, 0.003, 0.090, 0.056, and 0.115, respectively, and the Lave/Efron pseudo-R^2 was 0.63.

Likewise, logistic regression revealed for *P. washingtonensis* that the logit of the incidence was a function of inoculation date (DBH), average temperature of the leaf wetness period, and the concentration of germinable spores:

$$\ln\left(\frac{p}{1-p}\right) = -5.633 + 0.023*DBH + 0.125*T_{during\ a\ leaf\ wetness\ period}\ (°C) + 0.427*\frac{spores_{germinable}}{10^5} ml^{-1}$$

The standard error of the four parameters providing the elements of the function (−5.633, zero; 0.023, DBH; 0.125, temperature during a leaf wetness period; and 0.427, spores) were 0.773, 0.003, 0.033, and 0.153, respectively, and the Lave/Efron pseudo-R^2 was 0.43.

Discussion

The results of this study of inoculating commercial apple trees under natural conditions indicate that *N. perennans* and *P. washingtonensis* share certain features of their infection biology. Although both pathogens were able to cause infections at any inoculation date during the growing season, we found increasing infection levels resulting from inoculations within the last two months before harvest. This was more pronounced for 'Pinova' than for 'Braeburn'. Independently, we demonstrated this for both fungi also with the apple cultivar 'Nicoter' (H.H.F. Holthusen, PhD Thesis, submitted). These observations contradict previous trial results from the Lower Elbe region (Schulte 1997) and Sweden (Olsson 1965) but confirm reports from other countries on *Neofabraea* spp. (Edney 1958; Borecki 1961; Spotts 1985; Henriquez et al. 2008; Aguilar et al. 2017), *P. washingtonensis* (Sikdar et al. 2014), *Neonectria ditissima* (Xu and Robinson 2010) and *Sphaeropsis pyriputrescens* (Kim et al. 2014), thereby placing our findings in a broad context. The reasons

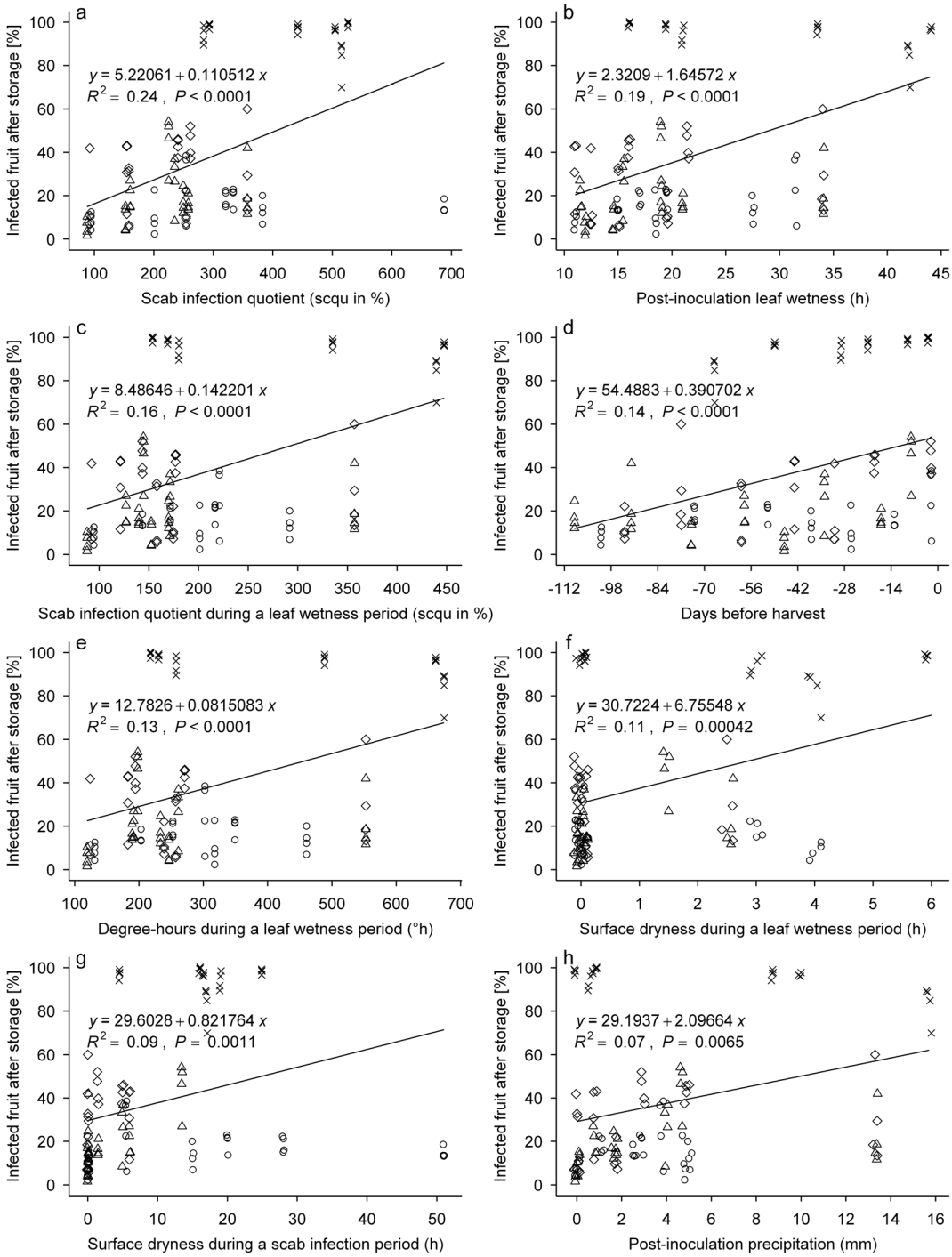

Fig. 3 Climatic and phenological parameters that influence the occurrence of fruit rot by *Neofabraea perennans* after storage following inoculation in the field. Single tree data from the experiments with cvs Braeburn in 2016 (○), Pinova in 2017 (×), Braeburn in 2018 (△), and Pinova in 2018 (◇) were used to calculate the linear regression curves. The following parameters were examined: (a) scab infection quotient according to Mills and Laplante (1954); (b) leaf wetness period; (c) scab infection quotient within a leaf wetness period; (d) time between inoculation and harvest; (e) temperature sum as degree hours above 0 °C within a leaf wetness period; (f) dry time within a leaf wetness period; (g) dry time within a scab infection period but after leaf wetness; (h) precipitation directly after inoculation

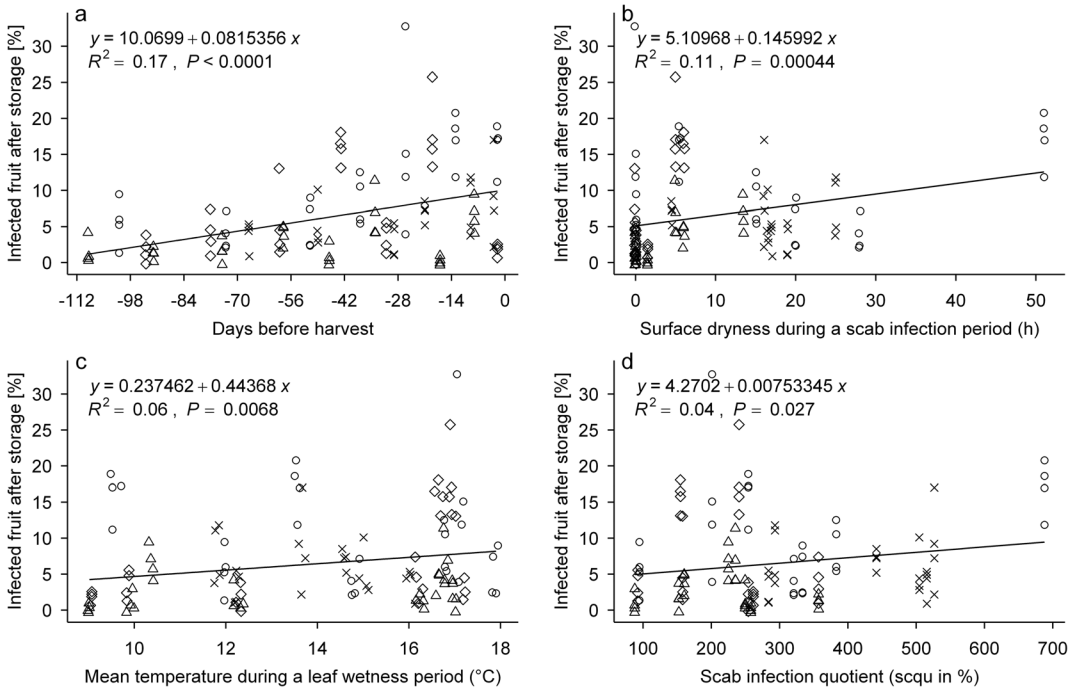

Fig. 4 Climatic and phenological parameters that influence the occurrence of fruit rot by *Phacidiopycnis washingtonensis* after storage following inoculation in the field. Single tree data from the experiments with cvs Braeburn in 2016 (○), Pinova in 2017 (×), Braeburn in 2018 (△), and Pinova in 2018 (◊) were used to calculate the linear regression curves. The following parameters were examined: (a) time between inoculation and harvest; (b) dry time within a scab infection period but after leaf wetness; (c) mean temperature within a leaf wetness period; (d) scab infection quotient according to Mills and Laplante (1954)

why fruit closer to harvest should become more susceptible to acquiring quiescent infections by so many storage-rot fungi are unclear but may be related to physical factors such as enlarging lenticels or microcracks in the fruit surface (Aguilar et al. 2017). The delayed outbreak of the active rot from quiescence after prolonged storage, on the other hand, may be due to processes of fruit ripening and senescence (Prusky and Lichter 2007; Wenneker and Thomma 2020), although little information is available on *Neofabraea* spp. and *P. washingtonensis* (Wenneker and Thomma 2020). However, the processes of germination and mycelial growth of *P. vagabunda* are strongly affected by the decrease of malic acid in ripening apple fruit, and the fungus itself is also able to increase the pH level of the fruit (Cameldi 2015; Cameldi et al. 2017).

Against this background, certain differences in cultivar susceptibility could be analysed on the basis of the 2018 data which permitted a direct comparison between Braeburn and Pinova. Cultivar differences have been described previously (Kaspers 1967). In view of the increasing susceptibility of fruit closer to harvest, we compared infection levels between equivalent inoculation dates before harvest. Although we found few differences for *N. perennans* or *P. washingtonensis* in the course of fruit development, 'Pinova' was more susceptible than 'Braeburn' on one or two inoculation dates (respectively) during the maturation phase within the last 6 weeks before harvest. Further work expanding over several seasons and more cultivars would be required to address this issue further.

Our statistical analyses revealed that *N. perennans* infections were influenced by several factors beyond the date before harvest. A positive correlation between leaf wetness duration and extent of infection which we observed for *N. perennans* is in line with previous reports for unspecified *Neofabraea* spp. (i.e. mainly *N. perennans*) by Schulte (1997), and for *P. vagabunda* by Giraud and Moronvalle (2012), but at odds with Henriquez et al. (2008) who found no such effect for *N. perennans* infections of pear fruit beyond the minimum requirement of a 30 min wetness period following inoculation. Our results for *N. perennans* are in line with the very high infection levels obtained in the wet 2017 season in our trial (Fig. 1b), and also with the high postharvest losses recorded in stored fruit from the

untreated controls of our 2017 fungicide spray trials (unpublished data). We observed no significant impact of leaf wetness temperatures on infections by *N. perennans* within the range of 9–18 °C, which supports the work by Giraud and Moronvalle (2012) for *P. vagabunda* but is at odds with the findings by Schulte (1997) for *N. perennans*.

Wetness duration and temperature are key factors influencing many fungal infections, as is particularly well-characterised for apple scab (*Venturia inaequalis*; MacHardy 1996). Not surprisingly, we found close correlations between fruit infestation levels by *N. perennans* and the apple scab infection quotient following inoculation, based on the matrix by Mills and Laplante (1954). As for *V. inaequalis*, brief dry spells of up to 6 h during a leaf wetness period did not negatively affect infections by *N. perennans*. This indicates that *N. perennans* could be modelled by modifying apple scab warning systems, which are well established. In France, degree-hours during a leaf wetness period was the factor best correlated with *P. vagabunda* infections (Giraud and Moronvalle 2012). Unfortunately, the apple scab infection quotient was not tested as a correlation factor in that study. However, an apparent contrast to *V. inaequalis* was that *N. perennans* and *P. washingtonensis* infections continued even after prolonged dry spells >16 h.

Linear regression analysis revealed a relatively weak positive correlation between post-inoculation precipitation and the resulting infestation level by *N. perennans*, but when using the generalised linear model this impact of post-inoculation precipitation became negative. Previous results on this issue have been similarly indecisive; Giraud and Moronvalle (2012) described a weak positive correlation for *P. vagabunda*, whereas Den Breeyen et al. (2020) saw no direct impact of the amount and duration of rainfall on *P. vagabunda*, and Schulte (1997) found a negative impact of post-inoculation precipitation on *N. perennans*. This can be interpreted such that heavy rainfall after inoculation may wash off conidia from the fruit surface, thereby reducing the share of spores capable of causing infection. In contrast, a prolonged leaf wetness without additional rainfall will favour *N. perennans* infestations. In general, the impact of rainfall is likely to be influenced by the inoculation method used. In our experiments we applied the spores directly to the fruit, whereas in the trials by Schulte (1997) they were washed from suspended fruit mummies onto the fruit by rain. An entirely different approach was taken by Giraud and Moronvalle (2012) who relied exclusively on natural infections.

In contrast, *P. washingtonensis* appeared to be little influenced by parameters other than days before harvest (see above), confirming results published by Sikdar et al. (2014) and Díaz et al. (2019), and the average temperature during a leaf wetness period following inoculation within the range of 9–18 °C. Both parameters were also used as the basis for our model to forecast *P. washingtonensis* infestation levels. To our knowledge, this is the first time that such a model has been attempted for this pathogen.

The concentration of viable inoculum remains a key factor for successful establishment of infections by any storage-rot fungus. For instance, Olsson (1965) needed 3.75×10^5 *N. perennans* conidia ml^{-1} for infecting apple fruit, whereas 3.75×10^4 conidia ml^{-1} were insufficient. Similarly, in our experiments we also recorded a reduced infection level when the share of viable conidia was low. Therefore, phytosanitation is important to keep inoculum levels in orchards down. For *N. perennans*, wood cankers associated with natural and artificial wounds are a key inoculum source (Edney 1956; Blumer 1960; Aguilar et al. 2019) and should be removed by pruning. Fruit mummies on apple cultivars are probably of lesser importance for *Neofabraea* spp. (Sharples 1959; Beer et al. 2015), but mummies on pollinator trees may be inoculum sources for *P. washingtonensis* (Weber 2011). The incidence of postharvest disease due to this fungus can be substantially reduced by pruning twigs of pollinator trees after flowering (Sikdar et al. 2018) or by removing fruit mummies during winter (Weber 2011).

An increasing susceptibility of maturing apple fruit to infections by storage-rot pathogens close to harvest necessitates pre-harvest fungicide applications at the very time when fungicides become less important for the control of foliar diseases, notably scab or powdery mildew, as shoot growth ceases. A reduction of such sprays can be achieved only if it becomes possible to predict the extent and species composition of rots likely to develop during storage. Recently a LAMP assay for the early detection of *N. perennans* in apples pre- and post-harvest has been described, although further validation is necessary (Enicks et al. 2020). Therefore, a modelling of the disease incidence as described in the present work may be an alternative risk assessment method at least for *N. perennans*.

Such a model should enable growers to deploy not only preharvest fungicide sprays but also more environmentally friendly postharvest options such as hot water treatments (Maxin et al. 2012, 2014) or drenching and fogging with fungicides (Aguilar et al. 2018; Ali et al. 2018) in a more targeted manner. The use of the scab infection quotient opens the chance of an easy integration into existing disease forecasting systems. Nonetheless, the models presented here need further validation in the region of origin and beyond before they can be widely used in practice.

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