Eur J Plant Pathol
https://doi.org/10.1007/s10658-024-02905-1

Differences in the seasonal development of perithecia by *Neonectria ditissima* on apple trees across Northern Europe

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Accepted: 15 June 2024

Abstract Ascospores discharged at rainfall and dispersed by wind can provide long-distance spread of the European canker fungus, *Neonectria ditissima*. Ascospores are produced by perithecia which are the sexual reproductive stage. Diffuse knowledge exists on the seasonal pattern of perithecium formation under different climatic conditions. Therefore, the development of perithecia was observed for several successive seasons at five sites in three Northern European countries. In Norway and Finland, ripe perithecia were commonly recorded throughout the year, and on individual cankers continuously for up to 28 months. In contrast, asexual reproductive structures (sporodochia) were confined to the growing season in both countries. In Northern Germany an average of 51% of cankers developed ripe perithecia by late winter, and perithecial senescence ensued in late spring. On average, ripe perithecia were present on cankers for 22 weeks. In contrast, sporodochia were observed all year round. The timing of perithecium maturation correlated with the number of days with > 2 mm rainfall in July–September. The presence of mature perithecia and sporodochia for different lengths of time in different countries has implications for regional disease management strategies.

Keywords Ascocarp · Apple canker · Conidia · Fruit tree canker · *Malus domestica* · *Nectria galligena*

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Introduction

European canker of apple, pear and quince as well as other broad-leaved trees is caused by *Neonectria ditissima* (Tul. & C. Tul.) Samuels & Rossman. Although the pathogen is present in all major apple-growing regions worldwide, there are differences in its importance due to the many regional factors influencing disease development, including climate and disease management strategies (Swinburne, 1975; Weber, 2014). Fresh wounds in the protective bark tissue are the entry point for infection. Both asexual macroconidia and sexually produced ascospores are highly infective (Latorre et al., 2002; Saure, 1961a), whereas microconidia play no relevant role in the infection biology (Wesche & Weber, 2023). In fruit production practice, scars caused by fruit picking and leaf abscission in autumn are among the most relevant wounds (Amponsah et al., 2015; Swinburne, 1975; Weber & Børve, 2021). Successful infections in autumn become visible as new cankers in the following spring. The first symptom is a brown discolouration as the outermost bark layer peels off (Weber, 2014). Sporodochia releasing macro- and microconidia are typically the first reproductive structures to appear on young cankers, followed by ascospore-producing perithecia at a later stage (Brandt, 1964; Saure, 1961a).

Two kinds of fruit rot, viz. blossom-end rot during the growing season and a storage rot after harvest, are produced by *N. ditissima* (Weber, 2014). Mummified fruit have been reported to harbour both sporodochia and perithecia in Brazil (Araujo et al., 2022), but only macroconidia from sporodochia were found on mummified fruit in North-western Europe (Weber, 2012).

The relevance of ascospores in canker epidemics is a controversial subject (Weber & Børve, 2021). Perithecia have been claimed to be rarely produced, or their ascospores to be of lesser importance, in relatively arid growing areas such as Chile (Carreño & Pinto, 1980; Latorre et al., 2002). In contrast, their release of ascospores has been credited with a major role in canker epidemiology in Northern European countries such as Northern Germany (Saure, 1961a) and the United Kingdom (Swinburne, 1975). The reasons for these different assessments are not entirely clear. This controversy may be due to a lack of data concerning the dynamics of perithecial development in response to climate, cultivars and growing practices. The natural potential of reaching new hosts is considerably higher for wind-dispersed ascospores which may be spread over distances of several hundred metres, than for splash-dispersed ascospores and macroconidia (Saure, 1961a; Swinburne, 1975). Therefore, the development of perithecia is a crucial part of the life cycle of *N. ditissima*, and we must assume this process to take place at variable intensity and at different times of the season under different climatic conditions (Amponsah et al., 2017; Munson, 1939; Swinburne, 1971; Weber, 2014; Wessel, 1979a).

The objective of our research was to assess the development of *N. ditissima* spore structures in several successive seasons under the different climatic conditions in Northern European apple-growing regions including Northern Germany (two locations), Norway (two locations) and Finland (one location).

Materials and methods

The main body of experimental work was carried out in 2017 to 2022. This included a documentation of developmental steps leading to perithecium maturation, the establishment of a key to permit identification of these steps, observations of new cankers for the incipient development of perithecia, and long-term observations of the same cankers on living trees and detached twigs.

Assessment of perithecium development

Based on observations and on previous reports (Kennel, 1963; Wessel, 1979a) a key describing perithecial developmental stages was proposed (Table 1). Each stage was documented by characterizing representative samples at different times of the season.

Table 1 Key to identify stages of perithecial development in *Neonectria ditissima*

Stage	Appearance on the surface of a canker
0a	No fructifications visible on the canker (Fig. 1a)
0b	Only white to pale yellow sporodochia with conidial production present (Fig. 1b)
1	Bright orange perithecial primordia, about 100 µm wide (Fig. 2a)
2	Enlarging carmine-red perithecia < 250 µm wide, no development of ostiole (Fig. 2b)
3a	Ripe red perithecia 300–350 µm wide with a darker-coloured apex or an opening (ostiole) (Fig. 1c, 2c)
3b	Ripe perithecia (as stage 3a) with white cirrhi containing ascospores (Fig. 2d)
4	Darkening brown or mahogany-coloured perithecia in senescence (Fig. 2e)

Size (width) of each stage was measured with a stereo microscope. Colour and appearance of perithecia were documented by photography. All developmental stages described in the key were identifiable with the aid of a standard × 10 hand lens.

Following removal and size measurement of perithecia of all stages, microscopic squash preparations were made in water to examine them for the presence of ascospores (Fig. 2f). The spore mass was then left in water for 24 h at 20 °C to allow for spore germination (Fig. 2g). For each stage, the contents of typical perithecia were examined at three different time points including at least five samples of each structure each time.

Perithecial development in Northern Germany

The two locations chosen for observations were the experimental orchard at the Esteburg Centre (53.51°N, 9.75°E) and a commercial orchard in Balje (53.82°N, 9.16°E). At the Esteburg orchard 10–15 new cankers were marked on 5-yr old trees of each of two cultivars (Nicoter and Fresco) growing in neighbouring plots under integrated pest management (IPM), and on cv. Fresco under organic management, in early autumn (September) of 2017, and observed weekly until May 2018. In each of the following seasons 20 cankers were marked and followed in each plot, except for 2021 when no suitable cankers were found on cv. Fresco under IPM and 40 cankers on the organic Fresco plot were used instead. All cankers had appeared earlier in the same season, and they were observed until the following late spring or summer in 2017–2019 and for about a year when starting in 2020 and 2021. The most advanced developmental stage in each canker was noted each time. In the 2018–2021 seasons the shares of each stage were also noted in cankers where different structures were present. In Balje a similar procedure was followed on cvs. Braeburn and Red Jonaprince, both under IPM. For each site and cultivar combination, the shares of cankers with developing and ripe perithecia were calculated as three or four replicates by grouping 3–10 cankers together. Weekly observations of shares of different developmental stages were combined to give monthly values for statistical analysis. When year and month were compared, the calculated incidence per cultivar was the replicate. From autumn 2017 the longevity of ripe perithecial structures on individual cankers was determined as the time between stages 3a and 4 becoming dominant, whereas from autumn 2018–2021 the duration of perithecial maturity was calculated from the shares of stage 3a or 3b.

Observations of cankers in Norway

Systematic observations of attached cankers were made in different experimental orchards at NIBIO Ullensvang (60.32°N, 6.65°E) and a commercial orchard in the vicinity. Occasional observations were also made in the Lier area in South-eastern Norway (59.79°N, 10.26°E).

In the experimental orchards at NIBIO Ullensvang systematic observations began in March 2018, i.e. after the majority of winter pruning and canker removal had been completed. Remaining cankers in different experimental orchards were observed at about weekly intervals. Sporodochia were also recorded where present. The cankers were located both on the rootstock and on the upper part of the trees. Snow cover and ice prevented access to some of the cankers in winter. The cankers available were observed throughout a two-year period until April 2020. In addition, new cankers were included, with observations usually beginning in autumn or late winter/spring. A total of 45 cankers was examined. Weekly shares of developmental stages were combined to monthly means.

In a commercial orchard close to NIBIO Ullensvang, cankers were marked in August 2019. In total 32 cankers were selected on 11 yr old trees (cv. Summerred) and 17 cankers on 7 yr old trees (cv. Red Gravenstein). The cankers were located on the tree trunks and lateral branches. The grower had scraped diseased tissues from cankers in the preceding winter, including 25% of the ones later selected on cv. Summerred. Assessments were conducted about weekly except for a five-week period during mid-winter. The final assessment was in March 2020.

In the Lier area cankers were observed three times in a commercial orchard. In total 15 cankers on cv. Julyred and 21 on cv. Summerred were marked in May 2018 and followed until October 2019.

Cankers collected from the experimental and commercial orchards were placed on a metal grid positioned on a mown grass surface a few meters from the meteorological stations at NIBIO Ullensvang in May 2018 and in Lier in January 2019. For observation with a stereo microscope, cankers were briefly taken to the laboratory. The share of each stage was quantified, except for stage 0b (sporodochia) which was assessed as present or absent. These assessments at NIBIO Ullensvang were performed weekly during the first four months, and at monthly intervals thereafter. The total number of detached cankers assessed in this experiment ranged from 23 to 40. Cankers contaminated by other fungi or showing no further development were discarded and replaced by newly cut cankers in May–June 2018, January–February 2019, September–October 2019 or January 2020. The detached cankers were grouped in three replicates, each containing about 10 cankers. In Lier the cankers were briefly observed by hand magnifier in 2019, and more thoroughly by a stereo microscope in February and June 2020.

Long-term observations in Finland

In Finland, observations were made in a commercial orchard comprising 50 yr old trees (cv. Lobo) grafted on rootstock A2 and located in Paimio, South-Western Finland (60.46°N, 22.69°E). The trees had cankers of different age which did not show any sporulating structures at the time of first observations in August 2017. From August 2017 until August 2018 the presence or absence of different stages was noted in 50 cankers in five tree rows. From August 2018 until the last observation in November 2020 the share of each developmental stage was also determined. Observations were made at about monthly intervals. Each time 40–70% of cankers had no reproductive structures. Sporodochia (stage 0b) were included in the total percentage. Tree row was used as replicate. Mean shares of different developmental stages for the cankers with visible fungal structures were calculated for each tree row before statistical analysis.

Weather records

Data from meterological stations at Esteburg (500 m from the experimental orchards), NIBIO Ullensvang (< 1500 m from the orchards), and Lier (30 km from the commercial orchard) were compiled for 2017–2022. Data for Freiburg (< 5 km from the Balje orchard) were provided by Meteostat (www.meteostat.net/de). The Paimio weather information was extracted from 10 × 10 km gridded data sets of the Finnish Meteorological Institute whose closest weather stations are 10 km and 25 km distant from the orchard (Venäläinen et al., 2005). The number of days with daily mean temperature above 5 °C per year was calculated as an indication of the growing season (Hansen-Bauer et al., 2017) and for activity of *N. ditissima* (Latorre et al., 2002). Further, the number of days with precipitation above 2.0 mm was determined, which corresponds to the rain event threshold in relation to *N. ditissima* spore dispersal used by Amponsah et al. (2017).

Statistical analysis

For each site, the shares of different perithecial stages were analyzed by the GLM procedure of SAS (9.4 SAS Institute, Cary, North Carolina, USA). Incidence data were arcsine-square root transformed before analysis. Only non-transformed values are presented in the figures. Mean values were separated by the Student Newman Keuls method at $P = 0.05$. Details of analyses are described in each experiment.

Results

The key of perithecial development (Figs. 1 and 2; Tab. 1) permitted a rapid identification of all stages in the field. Squash preparations of perithecia showed no differentiated ascospores in stages 1 and 2. Fully differentiated ascospores within their asci were found in abundance in stage 3, and these showed high germination rates of 90–100%. Ripe spores inside perithecia were often observed to have germinated *in situ* where they had failed to become ejected. In contrast, ascospores were seen only occasionally in stage 4 perithecia. At that late stage other fungi frequently covered the perithecial surface.

Meteorological observations

Different climatic conditions prevailed in the three countries where observations were made. These concerned both the annual mean temperatures and the total annual precipitation during the survey period of 2017 to 2022 (Table 2). With respect to *N. ditissima*, the total number of days per year with a mean temperature above 5 °C was 92 days shorter at Paimio (Finland), 78 days shorter in Lier (Norway) and 45 days

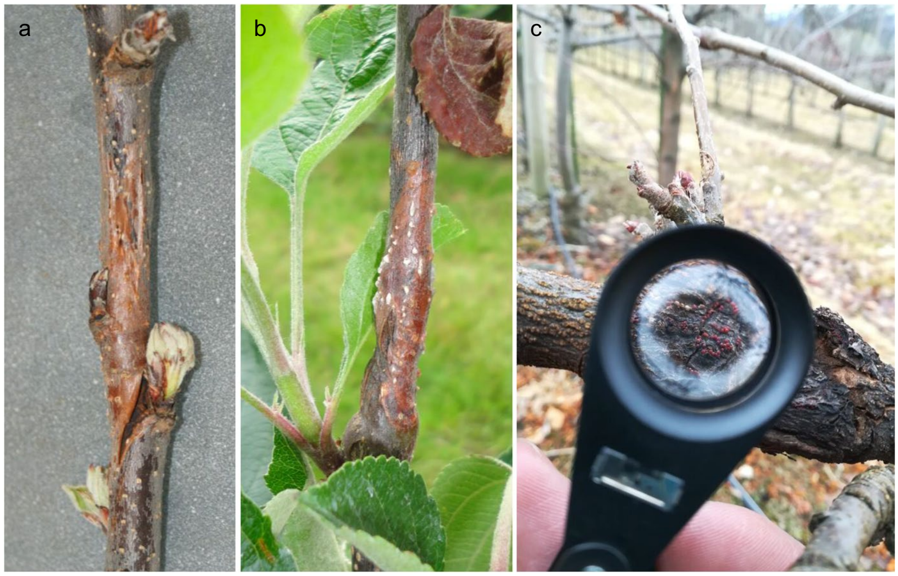


Fig. 1 Cankers caused by *Neonectria ditissima* on apple twigs. **a**. Epidermal discolouration and peeling as the first visible signs of an infection. **b**. Sporodochia of *N. ditissima* on an attached canker. **c**. Perithecial structures on attached cankers of *N. ditissima* as seen with a × 10 hand lens

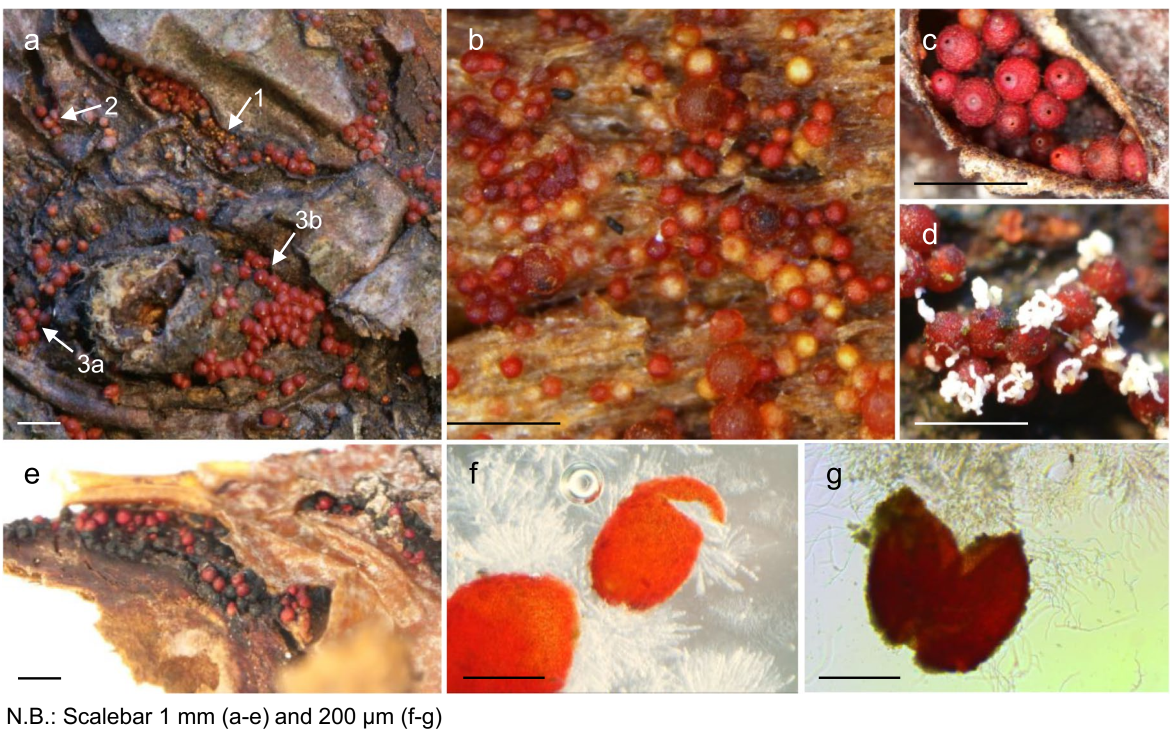


Fig. 2 Stages of perithecial development in *Neonectria ditissima*. **a**. Overview of a canker showing stages 1, 2, 3a and 3b. Examples of each stage are marked. **b**. Close-up showing mainly stages 1 and 2. **c**. Stage 3a showing the apical ostiole. **d**. Stage 3b showing white cirrhi of extruded ascospores. **e**. Darker perithecia putatively in the process of senescence (stage 4) in between stage 3a perithecia. f. Squashed ripe perithecium showing mature asci. **g**. Germinated ascospores after 24 h from a stage 3a perithecium. Scale bar 1 mm (a-e) and 200 µm (f-g)

Table 2 Average annual temperature (T, in °C) and cumulative annual precipitation (P, in mm) in five locations in Northwestern Europe in 2017 to 2022

	Paimio (FIN)		Lier (N)		Ullensvang (N)		Balje (D)		Esteburg (D)	
Year	T	P	T	P	T	P	T	P	T	P
2017	6.0	652	6.6	898	7.6	1664	9.9	1007	9.9	881
2018	6.6	462	7.0	499	8.4	1534	10.6	579	10.4	508
2019	6.6	738	6.7	1184	8.2	1542	10.4	726	10.7	697
2020	7.9	766	8.3	1062	8.8	2021	10.7	790	11.3	663
2021	5.9	653	6.6	720	8.2	1552	9.8	803	10.2	745
2022	6.4	579	7.3	689	8.2	1602	10.5	789	11.0	737
Mean	6.6	642	7.1	873	8.2	1653	10.3	782	10.6	705

shorter at Ullensvang (Norway) than at the Esteburg Centre (Germany) on average of six years (Table 3). The number of days above 5 °C varied between the years at each location. In Paimio the difference between the year with most and least days above 5 °C days was 30 days, in Lier 29 days, in Ullensvang 35 days, in Balje 43 days and at the Esteburg Centre 40 days. The number of days per year with precipitation of at least 2 mm was 128 in Ullensvang (average of 2017–2022) and this was higher than in all other locations (Table 3).

Development of cankers in Northern Germany

For the two Northern German locations, a total of 454 cankered branches was followed for 7–14 months in the five years of the study. Although a significantly higher share of cankers produced perithecia of any stage at Balje (63%) than at Esteburg (51%) across all five years of observations ($P = 0.0092$), there was no significant difference in the share of cankers developing ripe perithecia ($P = 0.1036$). Altogether 56% of cankers formed at least the initials of perithecia, 51% going on to produce ripe perithecia. The share of cankers with ripe perithecia ranged from 10% on cv. Fresco trees in IPM in the 2019 season at Esteburg to 100% on cv. Braeburn in 2017 at Balje. There were obvious variations in perithecium formation between the seasons analysed (Fig. 3). Thus, the proportion of cankers producing ripe perithecia was significantly higher in 2017 than in 2020 (Esteburg) or in all other years (Balje). In the 2020 and 2021 seasons, in which some of the cankers were followed for more than 12 months, a few of them developed a fresh crop of perithecia in the second autumn. These cases were not included in the totals in Fig. 3.

Because the two Northern German sites did not differ significantly in their overall developmental patterns in any of the years ($P > 0.05$), the five-year data from both sites were pooled for further analysis (Fig. 4). On a month-by-month basis, sporodochia dominated in September to December. During this time, rising shares of cankers with perithecium primordia (stage 1) and immature perithecia (stage 2) were observed, followed by a successive increase in cankers with ascospore-producing perithecia (stages 3a, 3b) from October onwards. These reached a

Table 3 Number of days with a mean temperature above 5 °C (T), and with precipitation > 2 mm (P) in five locations in Northwestern Europe in 2017 to 2022

	Paimio (FIN)		Lier (N)		Ullensvang (N)		Balje (D)		Esteburg (D)	
Year	T	P	T	P	T	P	T	P	T	P
2017	175	75	199	87	227	145	276	118	273	128
2018	202	58	201	46	229	111	261	83	263	69
2019	188	101	193	110	233	120	295	114	294	97
2020	205	103	222	104	262	149	304	104	302	85
2021	189	91	212	75	242	105	270	98	276	99
2022	196	79	213	76	244	136	285	103	303	92
Mean	193	85	207	83	240	128	282	103	285	95
			(+14)		(+47)		(+89)		(+93)	

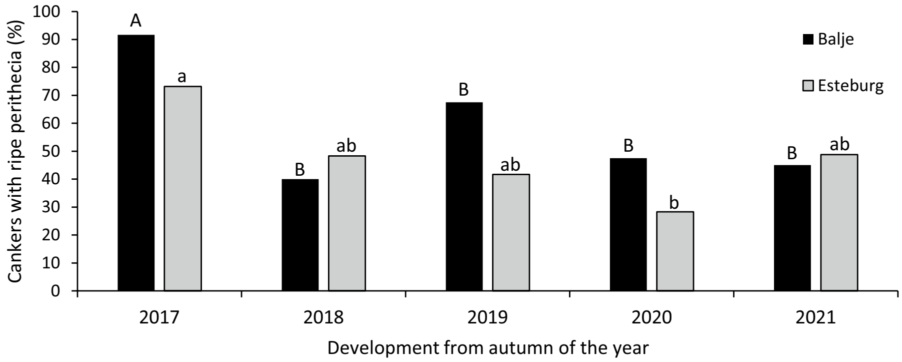
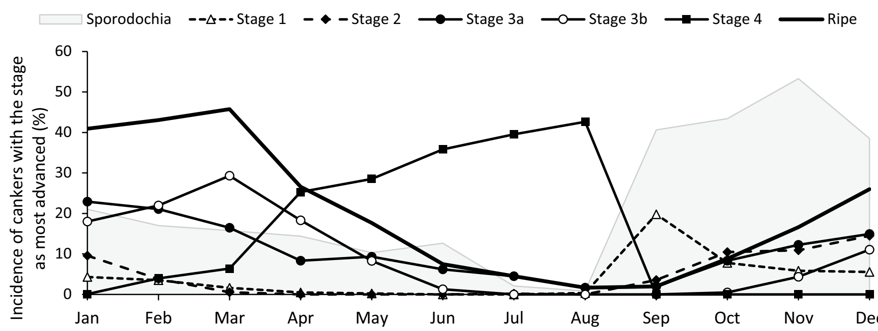


Fig. 3 Annual share of *Neonectria ditissima* cankers producing ripe perithecia on apple trees in Balje (black bars) and Esteburg (grey bars), Northern Germany. Means of 2–3 plots (cultivar and site combinations) with 10–40 cankers per plot at each location. Different letters within a location indicate significant differences according to the Student Newman Keuls method at P = 0.05

Fig. 4 Monthly mean shares of the most advanced stages recorded on *Neonectria ditissima* cankers on apple trees at two Northern German sites. Data are the means of 4–5 plots (cultivar and site combinations) with 10–40 cankers per plot, and up to five years' observations



plateau in January to March before gradually declining, giving way to senescing perithecia (stage 4).

The proportion of cankers featuring ripe perithecia as the most advanced stage was highest from October to April in each of the five years of the survey (Fig. 5). The earliest rise in perithecial maturation was recorded in the exceptionally wet summer season 2017 (211.8 mm precipitation from July to September at Esteburg, and 268.2 mm in Freiburg; data not shown), whereas the latest onset followed the exceptionally dry summer 2018 (77.4 mm and 142.9 mm, respectively).

Considering all cankers that permitted development of perithecia to ripeness within the observation period, the average time span of sexual reproductivity of individual cankers was 154 days, ranging from 17 to 462 days on individual cankers. The shortest duration of perithecial maturity was in 2018, whereas the longest ones were recorded in 2020 and 2021 (Fig. 6).

The date of autumnal onset of perithecium production was dependent on the weather during the preceding summer. The total number of days with > 2 mm rainfall in July to September of each year (Table 2) correlated well (Pearson correlation coefficient r = 0.79, P = 0.0065) with the day on which at least 25% of fertile cankers produced their first ripe perithecia (Fig. 7). The higher the number of wet days, the earlier the onset of perithecial maturation.

Weekly observations in a commercial orchard in Norway

Essentially the same experimental setup as in Germany was attempted in a commercial orchard near NIBIO Ullensvang from late summer 2019, except that in Ullensvang cankers of any age instead of current-season cankers were considered. On cv. Red Gravenstein 5 of 17 cankers and on cv. Summerred 12 of 32 cankers went on to develop perithecial structures.

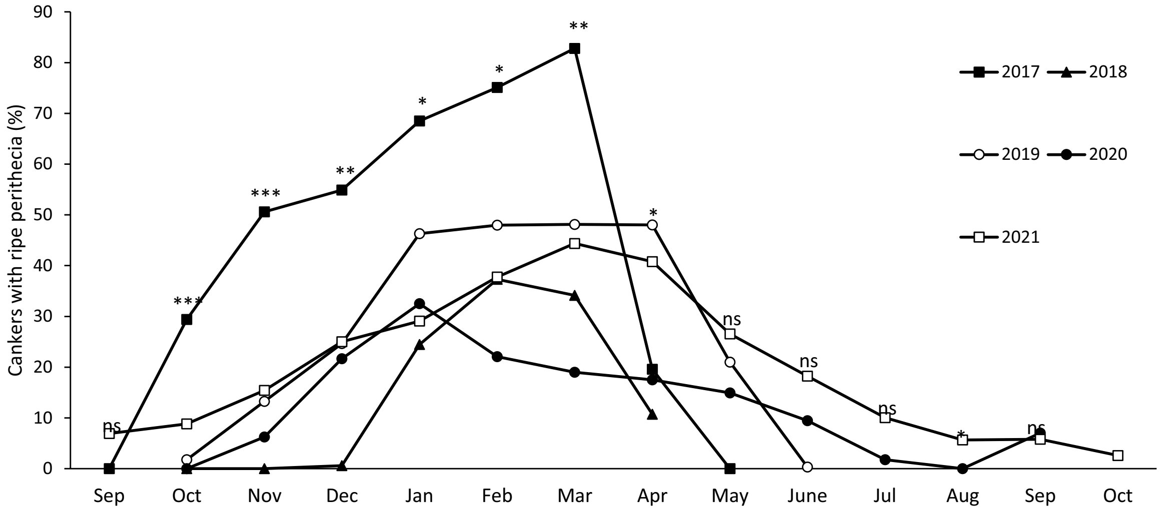


Fig. 5 Percentage of canker lesions of *Neonectria ditissima* on apple trees with ripe perithecia (both with or without cirrhi) as the most advanced stage as the means of 10–40 cankers on each of five (four in 2021) plots (cultivar and site combinations) at two locations in Northern Germany. Labels indicate significant differences between years at P < 0.05 (*), P < 0.001 (**), or P < 0.0001 (***), or non-significance (ns)

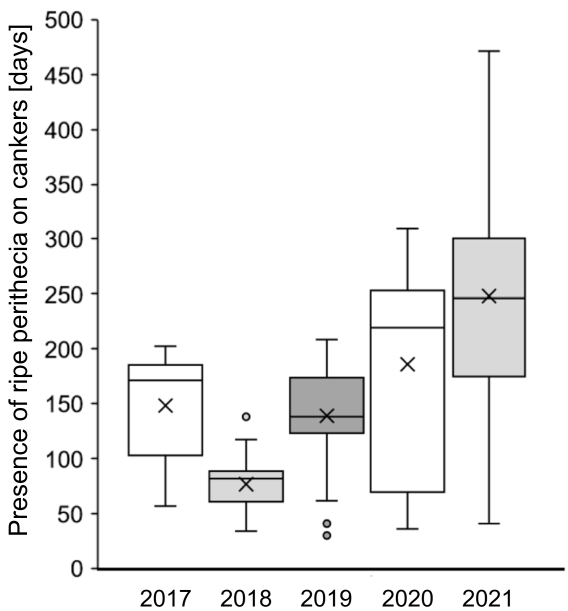


Fig. 6 Duration (days) in which individual cankers of *Neonectria ditissima* on apple trees had ripe perithecia (both with or without cirrhi) in 2017–2021. Mean of five (four in 2021) plots (cultivar and site combinations) at two locations in Northern Germany

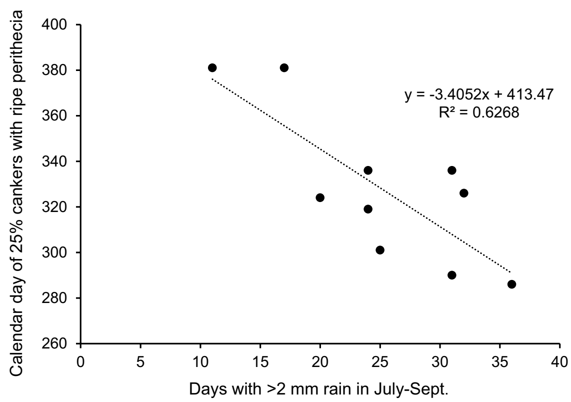


Fig. 7 Relationship between number of days with > 2 mm rain in July to September each year, and the numerical date by which at least 25% of cankers of *Neonectria ditissima* on apple trees had ripe perithecia in 2017–2021 at two locations in Northern Germany

Cankers were grouped in three replicates per cultivar before statistical analysis. Sporodochia were present during the first weeks of observation whereby a gradual decline was recorded from week 34 (67% of surface coverage) to week 38 (9%), at which point there was no significant difference to the rest of the season when sporodochia were absent. Perithecial primordia (stage 1) were confined to two weeks in March (week

10 and 11) in which their share was less than 10%. Developing perithecia (stage 2) were present in 15 of the 22 weeks of observation, being highest in weeks 38 and 39, i.e. in late September. Mature perithecia were present throughout the examination period, whereby stage 3a showed its peak in weeks 41–42 (early October) and stage 3b in week 3 (late January). Altogether, mature perithecia dominated from early October until March (Fig. 8). Significant ($P < 0.05$) differences over time were observed for all stages except perithecial primordia (stage 1).

Long-term observations of cankers

Longer-term observations of cankers were performed from spring 2018 to November 2020 at NIBIO Ullensvang on trees in experimental orchards (Fig. 9a) and on detached cankers (Fig. 9b), as well as on trees in Finland (Fig. 9c). Ripe perithecia were present throughout the whole period of assessment both in Finland and Norway, and both on detached and attached cankers in Norway.

At NIBIO Ullensvang cankers were followed on a range of different cultivars and in different positions in the trees. The monthly means of 2–3 years revealed an annual cycle in the sense that the largest share of cankers with ripe perithecia was recorded from October through to March followed by a gradual decline (Fig. 9a). Nonetheless, ripe perithecia were present on cankers both on rootstocks and on scion wood at every month throughout a two-year period. Cankers on the scion wood as well as on rootstocks produced sporodochia from June to August 2018 and from April to September 2019. No sporodochia were recorded in winter. Taking the two years together, the share of cankers with sporodochia was significantly higher on rootstocks (15.9%) than on the scion wood (10.9%, $P = 0.0030$).

In Lier ripe perithecia were present in 86% of the cankers in May 2018, the remainder producing only sporodochia. A year later 77% of the cankers bore ripe perithecia whereas the remainder had dried out with no visible sporulating structures. At the last observation in October 2019 all the active cankers had produced a new crop of perithecia (data not shown).

An analysis of detached cankers at NIBIO Ullensvang showed differences between the years (Fig. 9b). Sporodochia were observed from May to September 2018 and 2019 whereas ripe perithecia were observed all year round. In 2018 coverage by ripe perithecia was significantly higher in September to December than in May to August. In 2019 coverage by ripe perithecia remained high throughout, with a drop in February. Four of the detached cankers were kept for the entire period from May 2018 to June 2020. One of these cankers supported ripe perithecia throughout (Fig. 10).

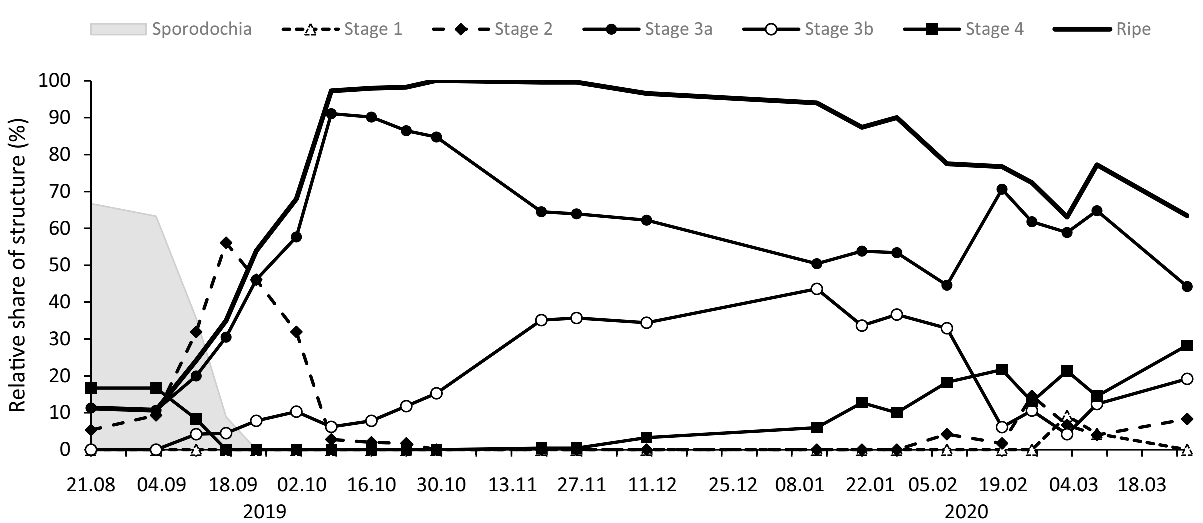


Fig. 8 Relative share in coverage of *Neonectria ditissima* spore structures at weekly intervals from autumn 2019 to spring 2020 in a commercial orchard with cvs. Red Gravenstein and Summerred in Ullensvang, South-western Norway

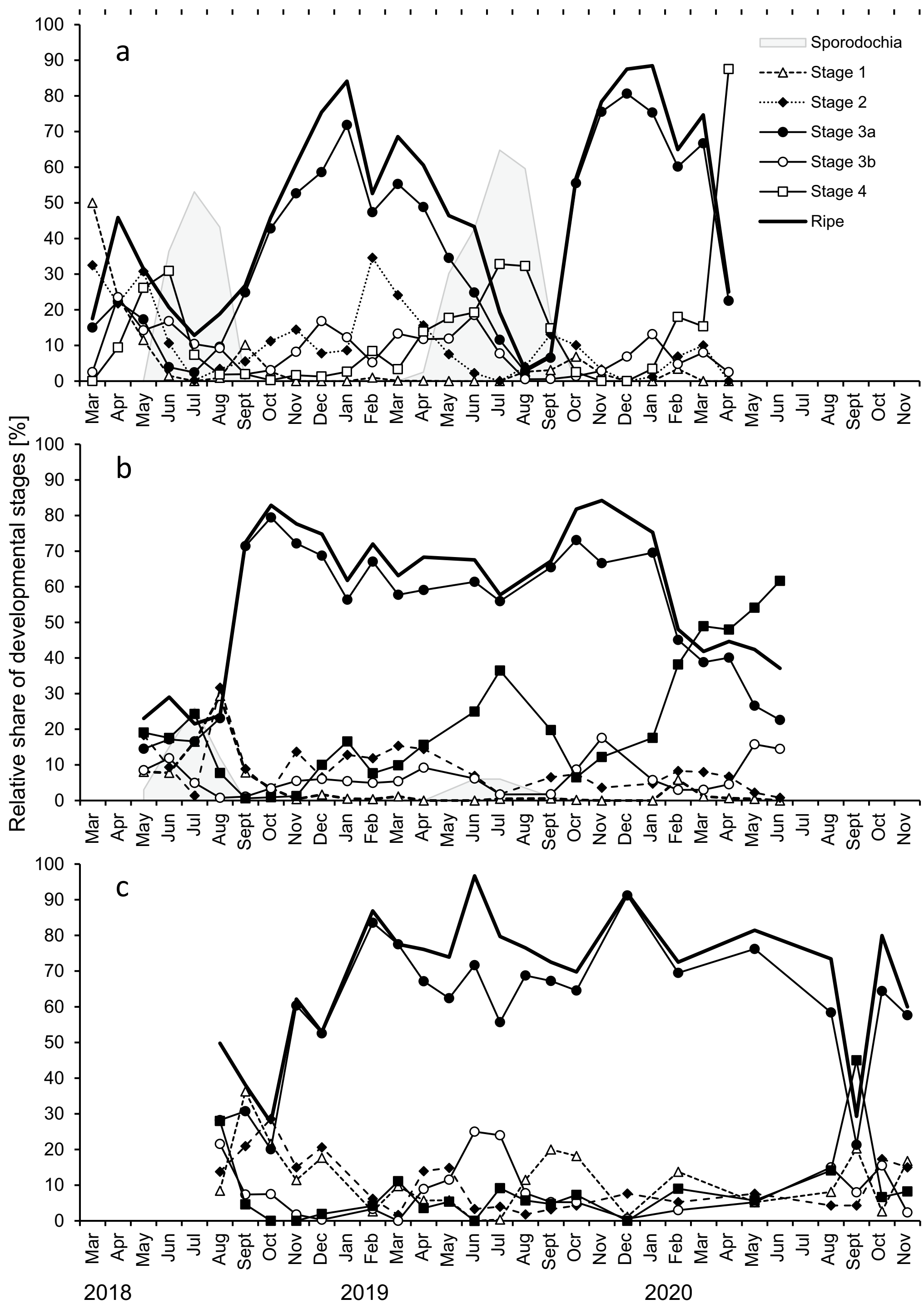


Fig. 9 Monthly mean of the relative share of coverage of perithecial development stages on cankers of *Neonectria ditissima* from March 2018 to November 2020. **a**. Attached cankers on apple trees at NIBIO Ullensvang (South-western Norway); mean of up to 45 cankers. **b**. Detached cankers from apple trees at NIBIO Ullensvang; mean of 23 to 40 cankers. **c**. Attached cankers on apple trees of cv. Lobo in Paimio (Finland); mean of 31 cankers

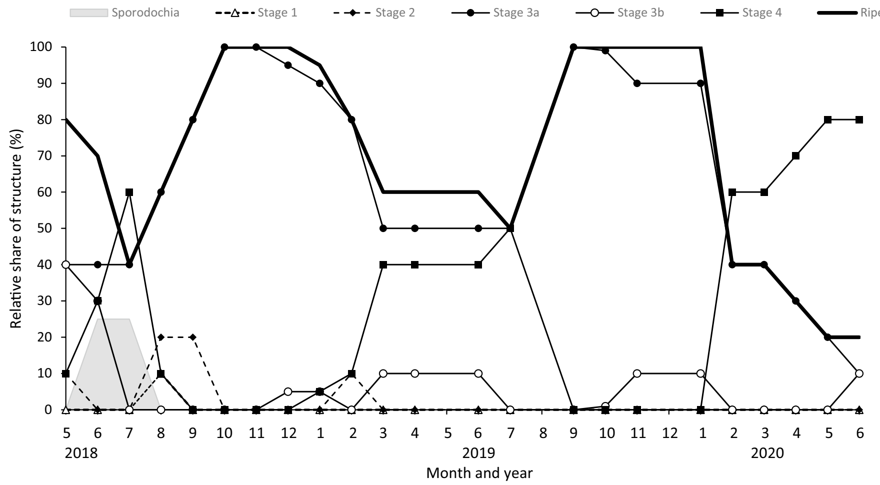


Fig. 10 Relative share in coverage of sporodochia and different perithecial developmental stages in a single detached *Neonectria ditissima* canker placed on a grid on a grassy surface area in South-western Norway from May 2018 to June 2020

In Finland the fate of attached cankers was followed in a 50-yr old orchard between August 2018 and November 2020, during which period 62% of the cankers developed perithecial structures. Ripe perithecia were present throughout the period at a coverage of 50–97%. Individual cankers harboured ripe perithecia throughout the survey period.

Discussion

The present work has revealed similarities as well as differences in the temporal patterns of perithecium development between Northern Germany on the one hand and the two Nordic countries, Norway and Finland, on the other. A shared feature between all three countries was that the development of most perithecia was initiated in autumn and that mature perithecia reached their peak some time in winter or spring, followed by a decline in summer. Further, in all three countries a proportion of cankers supported ripe perithecia continuously for more than 12 months, sometimes even more than 24 months. Nonetheless, there were quantitative differences in the rhythmicity of ripe perithecia throughout the year. In the Nordic countries the onset of perithecial senescence was retarded and ripe perithecia were present well into summer whereas in Northern Germany they senesced in spring or early summer. On the other hand, in Northern Germany cankers produced sporodochia and conidia for most of the year, whereas in Norway and Finland these were limited to a shorter period of the growing season. The consequence of such differences might be that ascospores play a larger, possibly a dominant role in the Nordic countries whereas conidia could be more relevant in Northern Germany.

In Northern Germany the dynamics observed in the present study – perithecia being present from late autumn until spring, sporodochia being present for most of the season – are broadly in line with previous long-term observations extending over several successive years (Kennel, 1963; Saure, 1961a). However, the strong extension of perithecium production into late spring was not previously reported. As an exception for Northern Germany, Wessel (1979a) described a pattern akin to the Nordic situation in which perithecia were found for most of the year in the single 1977 season.

There are heterogeneous reports concerning perithecial maturation, as indicated by ascospore release, from other regions. Munson (1939), working at Long Ashton near Bristol (South-West England, UK), reported a pattern similar to Norway and Finland in which ascospores were produced throughout the year but reached a peak from January through April. In Northern Ireland, a bimodal pattern was observed, with a major activity in spring and early summer and a minor one in autumn (Swinburne, 1971). In New Zealand, Amponsah et al. (2017) recorded both conidia and ascospores at any time of year provided that sufficient moisture was available.

Which environmental factors might be responsible for such differences? Saure (1961a), working in Northern Germany, obtained evidence of a correlation between extended wet periods in summer to early autumn and the onset of perithecium formation. In the present work we were able to confirm this effect and quantify wetness as the cumulative number of rainy days from July to September. Following a different approach, Kennel (1963) placed detached twigs with cankers not yet producing perithecia in damp, protected conditions close to the soil surface in spring and observed the onset of perithecial production in summer whereas twigs attached to trees would not produce them until late autumn. In terms of prolonged moisture requirements for fruit-body development, we may liken perithecia of *N. ditissima* to basidiocarps of agarics and boletes which normally develop in autumn as the daily periods of saturated moisture extend, but which do occasionally produce a crop even in mid-summer during prolonged spells of wet weather at least in northern and western Europe.

Temperature is likely to play an additional role, higher temperatures favouring the production of sporodochia whereas lower autumn temperatures seem to trigger perithecial formation (Wessel, 1979a). Between Northern Germany and Norway / Finland there were differences of 2–3 K in yearly mean temperature throughout the period of study. This ties in with Latorre et al. (2002) who demonstrated that ascospores are able to germinate at lower temperatures, and have a lower temperature optimum, than conidia.

Such differences between perithecium and sporodochium maturation might make an impact on fruit production practice. Whilst there is strong agreement that leaf scars constitute one of the most important entry points for *N. ditissima*, views on the timing of such infections are controversial. Most authors have considered fresh autumnal leaf scars to be critical (e.g. Dubin & English, 1974; Kennel, 1963; Latorre et al., 2002; Swinburne, 1975; Weber, 2014), although others have favoured bud scale scars or leaf scars in spring (Marsh, 1939; Swinburne, 1971). However, in all commercially managed orchards at least in temperate zones, intensive spray regimes against apple scab (*Venturia inaequalis*) have to be conducted in spring, and most of the fungicides used – copper salts, captan, dithianon, dodine – also have an effect against *N. ditissima* (Saure, 1961b; Swinburne et al., 1975; Cooke et al., 1993; Cooke, 1998). Therefore, scars in spring are unlikely to play a major role in the epidemiology of *N. ditissima* in such orchards. In contrast, no sprays are conducted in autumn against any pathogenic fungus other than *N. ditissima*, and the high efficacies of fungicide sprays at leaf fall leave no doubt that leaf scar infections at that time are highly relevant in practice (Weber & Børve, 2021). The timely onset of perithecial ripening in autumn appears to be the main chance for *N. ditissima* to harness its ascospores for infections of leaf scars in fruit orchards – in addition to pruning wounds caused by orchard management in winter (Alves & Nunes, 2017; Marsh, 1939; Xu et al., 1998). In this line of thought, it is relevant that ascospores are able to germinate at temperatures below 10 °C more readily than conidia (Latorre et al., 2002). Wounds on the fruit spurs caused by fruit picking are another highly relevant point of entry (Amponsah et al., 2015), but these are caused 1–3 months before leaf fall and therefore fall into a period when ripe perithecia are at their lowest ebb at least in Germany, but macroconidia are abundant.

In Norway we found that detached perithecium-bearing cankers placed on the ground continued to support mature perithecia in a manner substantially similar to cankers still attached to twigs. This observation confirms previous reports by Saure (1961a) and Berrie et al. (2008). The issue strikes at the heart of a long-standing controversy concerning the removal or non-removal of infected material from the orchard after canker pruning. Our pragmatic advice in recent years has been such that larger branches, trunk sections or even entire tree trunks must be removed, stored dry and burned whereas small twigs with young cankers may be dropped to the orchard floor on the assumption that the material will have decomposed or become colonised by other fungi before perithecia can mature. Given that numerous ascospores (or conidia) per wound are required to initiate a canker infection (Xu et al., 1998), the risk of a slight ascospore discharge from small twigs on the ground seemed tolerable if these were left behind during the main canker pruning period in spring and early summer.

Berrie et al. (2008) found that pruned twigs dropped on the alleyway decomposed more quickly than twigs on the vegetation-free surface under the trees. They also showed that perithecia continued to be present on detached twigs for at least 16 months after pruning, or for 12 months if the pruning material was shredded. Our observations of continued perithecial maturation for more than 12 months in Norway and 24 months in Finland highlight potential risks associated with detached cankers in the orchards in these countries.

Inoculum for autumnal infections of leaf scars and other wounds will be present as conidia and/or ascospores in all regions where canker is a relevant disease. The dispersal mechanisms differ between these two spore stages, but ascospores failing to clear the ostiole will form a deposit on the outside of the perithecium and can still be distributed by water splash. Indeed, the number of splash-dispersed ascospores may be considerably higher than those actively discharged and dispersed by wind (Wessel, 1979b). Even without ascospores, water running down along the tree surfaces during rain events may contain conidium concentrations exceeding 10^4 ml^{-1} (Butt et al., 1994; Graf, 1975), i.e. well above minimum concentrations needed for leaf scar infections (Bennett, 1971). The input of ascospores into the water runoff will increase inoculum density. In this sense, ripe perithecia are to be taken seriously as a contributing factor to within-tree disease development, in addition to the long-distance dispersal of ascospores between orchards. If ripe perithecia are present all year round in Scandinavia, as compared to much shorter periods in Northern Germany, there is a greater need to collect all pruning material from the orchard floor. Apart from that, both spore stages may be treated along similar lines: canker pruning and appropriate autumnal fungicide sprays remain the most critical measures against both.

Acknowledgements Norwegian apple growers made the project fund available through the Research Council of Norway (project no. 269209). The authors thank growers in Germany, Finland and Norway for providing access to the commercial orchards, the technicians at NIBIO Ullensvang for help with the experiments and Stefanie Kutz, Esteburg, for processing German observations into a suitable format.

Author contribution All authors contributed to the study conception and design. The first draft of the manuscript was written by Jorunn Børve and Roland W. S. Weber. All authors have read and approved the final manuscript.

Funding Open access funding provided by Norwegian Institute of Bioeconomy Research. The authors declare that they have no financial interests.

Declarations

Human and animal rights and informed consent No human and/or animal participants were involved in this research.

Conflict of interest The authors declare that they have no conflict of interest.

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