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Spore production and beetle visitation on the polypore *Fomitopsis pinnicola*

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Abstract:

Insects and fungi are important parts of dead wood systems. Polypores, the main structural decomposers of dead wood, have fruiting bodies frequently visited by insects. This could potentially be important for spore dispersal, but few studies have investigated whether insect visitation correlates with spore production.

In this study nine time-lapse cameras were used to register beetle visits on *Fomitopsis pinicola* in Nordre Pollen and Østmarka nature reserve. In addition passive spore traps were used to monitor spore production for five polypores in Nordre Pollen once a month, from May to August to see how visiting rates of beetles related to spore production, time of day, air humidity and temperature.

Spore production was at its peak in May, followed by June. No spores were found in August and only two polypores released spores during July. Activity of 4 beetle species was estimated from 51 852 high quality images and spore production was a good predictor for occurrence of beetles in May.

Contents

1 Introduction:

Boreal forests are important ecosystems that harbors large amounts of species (Hongve 1999, Patriquin et al. 2007). A large portion of the biodiversity in forests is connected to dead wood (Jonsson et al. 2005), with insects (Muller et al. 2002) and fungi (Norden et al. 2004) making up the large majority of the species. Both can affect forest dynamics through their involvement as decomposers, which is well documented (Ulyshen 2016) (Boddy and Watkinson 1995). Fungi is the most important decomposer in forests, followed by bacteria and insects (Weslien et al. 2011). Few studies have been conducted on the interactions between either of the three groups, even though these interactions could potentially influence species abundance, distribution and decomposing (Ulyshen 2016).

Fungi can break down the compounds cellulose and lignin which few other organisms are able to do, making them important and efficient decomposers of dead wood (Boddy and Watkinson 1995, Talbot et al. 2008). Polypores are the functionally most important, and most abundant fungi in these dead wood systems (Heilmann-Clausen and Christensen 2004, Kebli et al. 2011). Polypores have fruiting bodies that produce large amounts of spores on the hymenial surface, and these are frequently visited by insects (Johansson et al. 2006). The insects could be eating spores, the hymenial layer, or other visiting insects, but what species visit, how often they visit and what they do there is not well known (Hågvar and Okland 1997, Schigel 2012). The visits could have nutritional value for the insects, but they could also be important for the fungi's ability to spread its spores to new substrates (Jacobsen et al. 2018b). Fäldt (1998) found that insects are drawn to the smell of certain compounds secreted by polypore fruiting bodies during periods of heavy sporulation (Fäldt et al. 1998).

Jacobsen (2017) found that 54% of beetles that landed on freshly cut aspen wood carried DNA of wood-decomposing fungi indicating that they may act as targeted dispersers (Jacobsen et al. 2017). In a later study, they saw that the same beetles had a similar specialization in their network of fungi to that of insect seed-dispersers

(Jacobsen et al. 2018a). The visiting behavior from beetles has previously been viewed as mostly neutral or disadvantageous for the fungi, as beetles have been observed eating the hymenial layer of polypore, but considering the recent findings by Jacobsen, fungal grazing by beetles could have potential benefits for the fungus (Guevara et al. 2000).

Studies of insect activity on fungal fruiting bodies has previously been done using manual observations (Hågvar 1999), but this is tedious due to the relative scarcity of visits to the insects nocturnal activity. In recent times camera technology has been used to gather data for species interactions, particularly in mammals and recently in some insect studies (Steen and Barmoen 2017, Bjerge et al. 2021). A recent study by Ferkingstad (2020) found that camera monitoring could work as a great supplement to traditional observation methods when studying insects visiting polypores. It allows for data collection over large periods of time without the need for an observer physically present and enable estimation of visitation frequency which is highly important for the insects potential effect on spore dispersal (Ferkingstad 2020).

The polypore *Fomitopsis pinicola*, is a brown-rot polypore that is common all over Norway, although it is particularly common in older spruce forests due to these forests often having more dead wood (Komonen 2003). The fruiting body is characterized by its red-colored band around the side of the spore cap and can remain for decades under optimal conditions (Hågvar and Okland 1997). It also has a relatively season dependent spore-production rate, and it can produce many spores that are spread with the wind (Nuss 1986). Its relatively large fruiting bodies are frequently visited by insects and given its widespread distribution this polypore is an ideal study organism and will therefore serve as the study organism for my thesis (Hågvar 1999).

My goal will be to observe how visiting patterns by beetles on this polypore change with its sporulation cycle over multiple months. I will do this by using time-lapse cameras to photograph the fruiting bodies in parallel with measurements of spore production. In order to explore this relationship my study will aim to answer the following questions: “How does sporulation change with month, time of day, air humidity and temperature?” and “How does this compare to the changes in visiting rates by beetles throughout the

day?” Furthermore, since use of cameras is a relatively new technology for investigating insects on polypores, I wanted to compare my study with the study we build upon (Ferkingstad 2020) and evaluate improvements. Therefore an additional question was added: “How did the image quality from the cameras change when compared to last year?”.

I predict that *F. pinicola* has higher sporulation rates during the night, as the lack of sunlight could provide less harmful situations for the spores since they are not damaged by UV-rays (Fourtouni et al. 1998). Looking at findings from Ferkingstad (2020) and Hågvar (1999) who both found more beetles visiting during nighttime I also expect to observe this pattern (Hågvar 1999, Ferkingstad 2020). Furthermore, I predict a positive relationship between spore production and beetle frequency based on these findings.

2 Materials and methods:

2.1 Study area:

Two main nature reserves were used for this study. The first was Norde Pollen, and the second was Østmarka (Figure 1). Nordre Pollen is the youngest reserve having been established in 2005, but it has remained free from forestry and other intensive land use since 1982 (Miljødirektoratet 2005). Østmarka was established in 1990 but had been protected since the 1960s (Miljødirektoratet 2002).

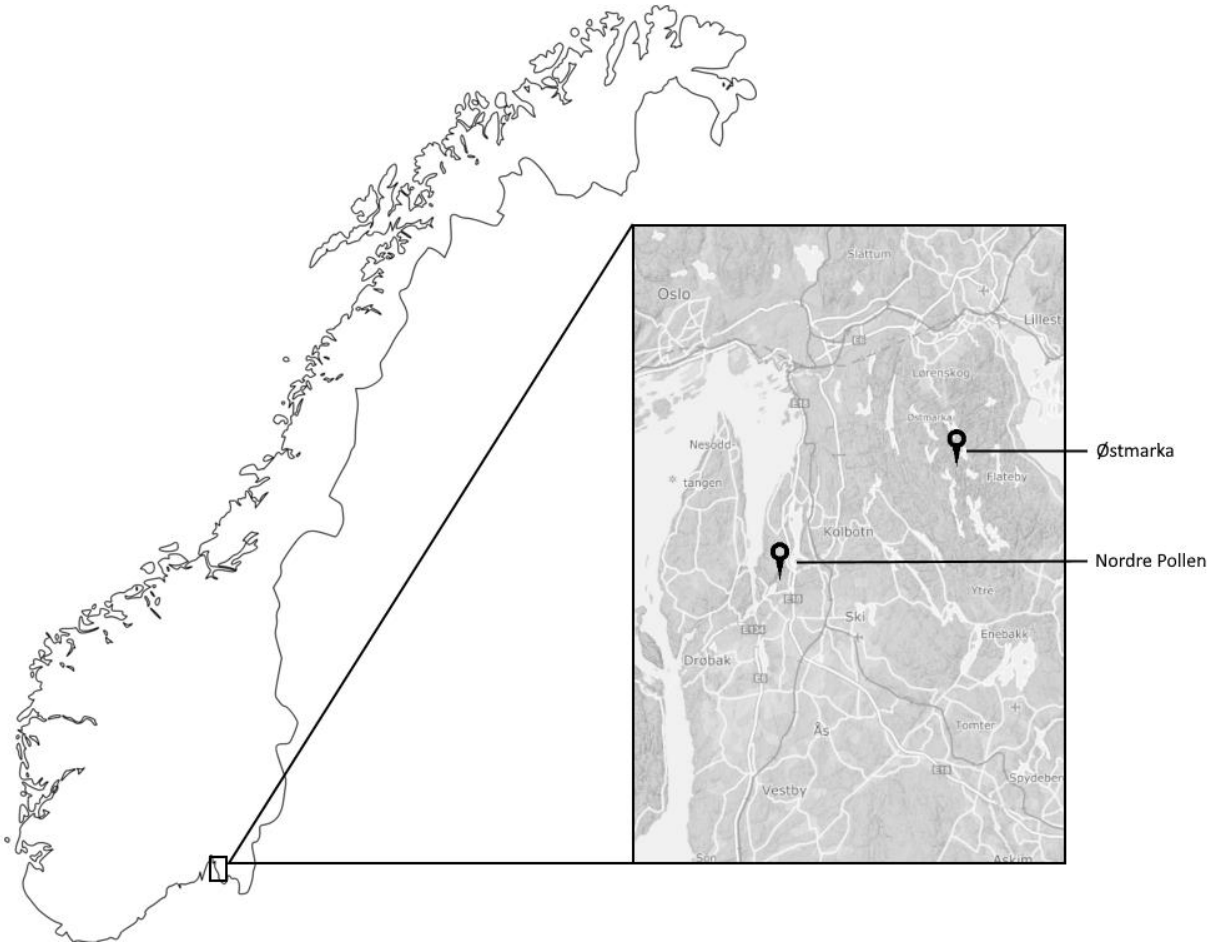


Figure 1. Locations of both study sites used for monitoring *F. pinicola*, Østmarka and Nordre Pollen.

2.2 Experimental design – cameras:

I initially planned to use 12 time-lapse cameras of the model Wingscapes® TimelapseCam Pro, WCT-00126, each with a corresponding camera mount, used to attach the camera beneath the polypore (Figure 2). However I ended up with nine cameras total (four in Nordre Pollen and five in Østmarka), as a result of two cameras malfunctioning and one being stolen during the study period. Due to the need to attach the camera in an upward-facing position the number of suitable polypores in each area were limited. Above the camera lens was a rubber seal with a plastic plate on top, intended to prevent water from clouding up the photos. The cameras have a battery life of around 1.5 months and are resistant to harsh weather, allowing them to run for long

periods of time. They have a strong flash, allowing them to take photos during night and can record the temperature at the time of each photo.

Figure 2. Setup showing the time-lapse camera attached beneath a fruiting body of F. pinicola using a custom mount. Photo: Tone Birkemoe

Six fruiting bodies of *F. pinicola* in each study area were selected and a camera was mounted to the stump they were growing from. The camera lens was fitted 20 cm away from the polypore, and the entire hymenial surface had to be in frame. The cameras were set to take one picture every ten minutes and store them on an SD card. The cameras were running from April 28th to September 2nd at Nordre Pollen and from April 17th to September 18th in Østmarka. During this time, I visited the sites twice to back up data and change batteries.

2.2.1 Initial image handling:

At the end of the season, all the photos were downloaded from SD-cards to an external hard-disc for image handling. The goal of the initial image handling was to automatically remove photos with a quality too low for detection of beetles. I randomly selected a sample of 1000 photos from each site. From these photos I manually identified 100 “high-quality” photos, defined as a photo where the hymenium of the polypore was so clear that insects could be identified down to family level. I then used a python script (Appendix 1) that calculated the Variance of Laplacian (VL) of each image. The

Variance of Laplacian is an approximation of how blurry a photo is, estimating image quality using an edge detection algorithm (Hoye et al. 2021). Using the sample of high-quality photos as a base, the script would then calculate a VL threshold value for high-quality photos and filtering out all that fell below this value. Each individual site was handled separately with the script to ensure more accurate threshold values. After filtering out all the low-quality photos from my data set, I extracted every sixth image (e.g., 12:00, 13:00 etc.) for manual annotation work in order to reduce bias towards the observations in the photos. I used a digital annotator named VGG Image Annotator (VIA), which allowed me to scroll through sets of images and manually select areas that contained visiting beetles in each photo. This was then exported into a .csv file. During the manual annotations, all beetles visiting were identified to the lowest possible taxonomic rank. However, the very high occurrence of *Gyrophaena boleti* living in the pores of the fruiting bodies was only annotated in clusters of 10 or more individuals. The reason for this is the assumed low impact that *G. boleti* has on spore dispersal, as they don't usually migrate between different fruiting bodies, living their lives withing the pores of the polypore (Hågvar 2018).

2.3 Experimental design – spore measurements:

I measured spore production on the four polypores with cameras in Nordre Pollen on four dates throughout the summer at the following dates: 20.05, 13.06, 20.06, 23.08. I took measurements every three hours throughout 24 hours to cover differences between day and night, resulting in 8 measurements per polypore. I used a passive spore trap following Norros and Veera, et al. (2012), in which three 1x1 cm squares of plastic were attached with pins to the hymenium of the polypore (Figure 3) to gather spores (Norros et al. 2012). After three hours had passed, they were removed and transferred individually to Eppendorf tubes and replaced with new squares. In addition, the hymenophore area of the polypores was measured to observe differences in size. During all trips, except the first, I measured temperature and relative air humidity using a hygrothermometer, to see what effect these factors could have on the sporulation.

Figure 3. Setup showing the plastic squares attached beneath a fruiting body of F. pinicola using pins. Photo: Tone Birkemoe

2.3.1 Spore counting:

Initially, each sample with plastic squares was added 400 µl of isopropanol to dilute the sample, herein referred to as the dilution factor. This amount was reduced in July and August due to low spore concentrations (300 µl and 200 µl of isopropanol respectively). Each sample was then vortexed and placed in a centrifuge at 1500 rcf for 30 seconds, after which 60 µl of the sample was put into a tube along with 60 µl of the staining compound cotton blue. 20 µl of the dyed sample was then placed into a Neubauer haemocytometer and the spores were counted in a microscope. The number of spores was then divided by the dilution factor which was assumed to be equal to spore production per 3 cm^2 area of the hymenium of the polypore using this formula:

Concentration (Spores/ml) = (Number of spores * 10 000)/(Number of squares in the haemocytometer (16) * (Dilution factor).

This number was then divided by the number of plastic squares (3) to get the approximate spore production per cm^2 for each polypore.

2.4 Statistical analysis:

All analyses in this study were conducted in RStudio version 1.4.1103. Analysis of which variables influenced spore production was done with a simple Anova test. The variables examined were month, time of day (in three-hour intervals), temperature and air humidity, and how each of them interacted with spore production by separately. The spore variable had a large span between high and low measurements, so the variable was transformed logarithmically. For one of the analyses, I also grouped times into day (06:00-20:59) and night (21:00-05:59) to see if spores were connected to the day-night cycle. For temperature and humidity, only the month of June was used since no measurements of them were made in May.

For how beetle visits related to spore production, I decided to use a General Linear Mixed Model for analysis. My response variable was the number of observed beetle species per ten minutes. This variable was not normally distributed, which is the reason why I went with a generalized model, since it handles non-normally distributed variables (Zuur et al. 2009). I wanted to compare this to the variables of spore production, temperature, and hour of day, however due to only having data on spore production from a single 24-hour period each month, assumed the spore numbers to be representative for each day the week the spore measurements were taken. In addition, only data from May and June was used, as the spore numbers were too low the following months. The data of beetle visits can be categorized as counting data, so I assumed it followed a Poisson distribution. Poisson distribution assumes that the variance and mean of the data set are equal, which is rarely the case for ecological data. However, in my dataset the variance and mean were almost identical, allowing me to utilize Poisson distribution without issue (see Appendix 2 for model diagnostics). I then decided to go with Mixed Model to account for the random effects (Henderson Jr

1982). Within the model, both the three-hour intervals used for spore measurements (Timeframe) and the polypore itself (Site ID) was categorized as random effects.

3 Results:

3.1 Spore production

Spore production varied between the months (Table 1). All four fruiting bodies produced spores in May and June with the highest production in May. In July spores were only found on 2 polypores, and in August no spores were recorded (Figure 4).

The number of spores produced in May and June did not vary between the eight time-intervals during the 24-hour cycle. (Figure 5, Table 2). This was also the case when the time was sorted into day and night (Table 3).

Spore production did not vary with temperature (Table 4) or humidity (Table 5) in June which was the only month this analysis could be carried out.

Table 1. Summary of Anova test showing the effect of month (May-August) on spore production (per cm^2 per 3 hours, log10-transformed) in four *Fomitopsis pinicola*- fruiting bodies on spruce snags in Nordre Pollen nature reserve, Norway.

	Df	**Sum sq**	**F value**	**P-value**
Month	3	$3.4*10^{11}$	6.12	<0.001
Residuals	124	$2.3*10^{12}$		

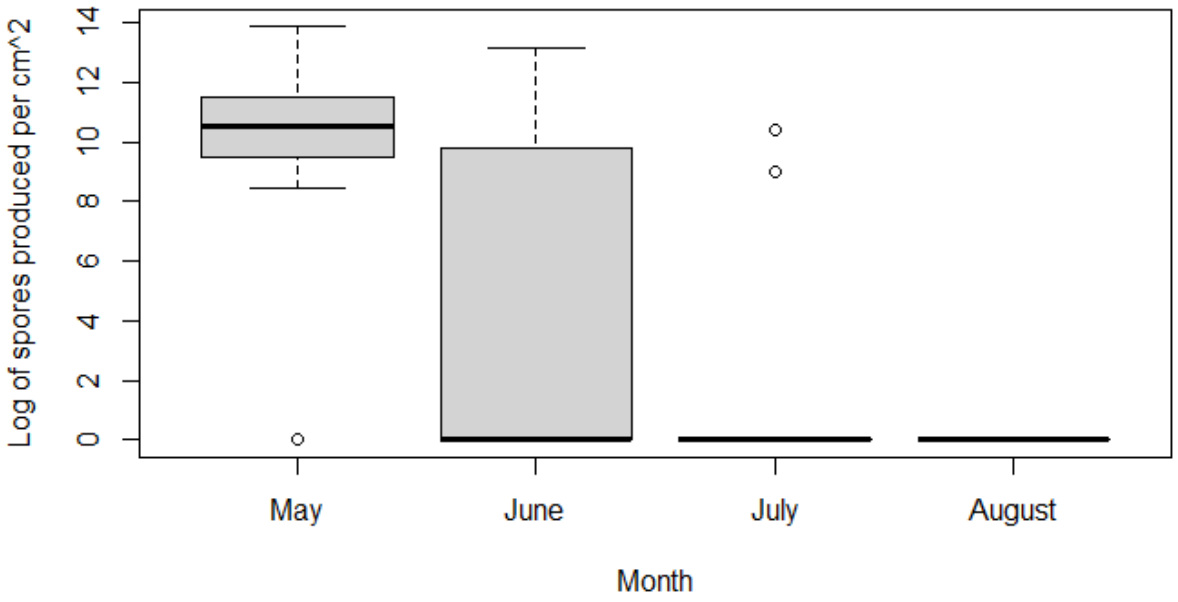


Figure 4. Boxplot of spore production (per cm^2 per 3 hours, log10-transformed) in four *Fomitopsis pinicola*- fruiting bodies from Nordre Pollen nature reserve, Norway, measured during one diurnal cycle each month.

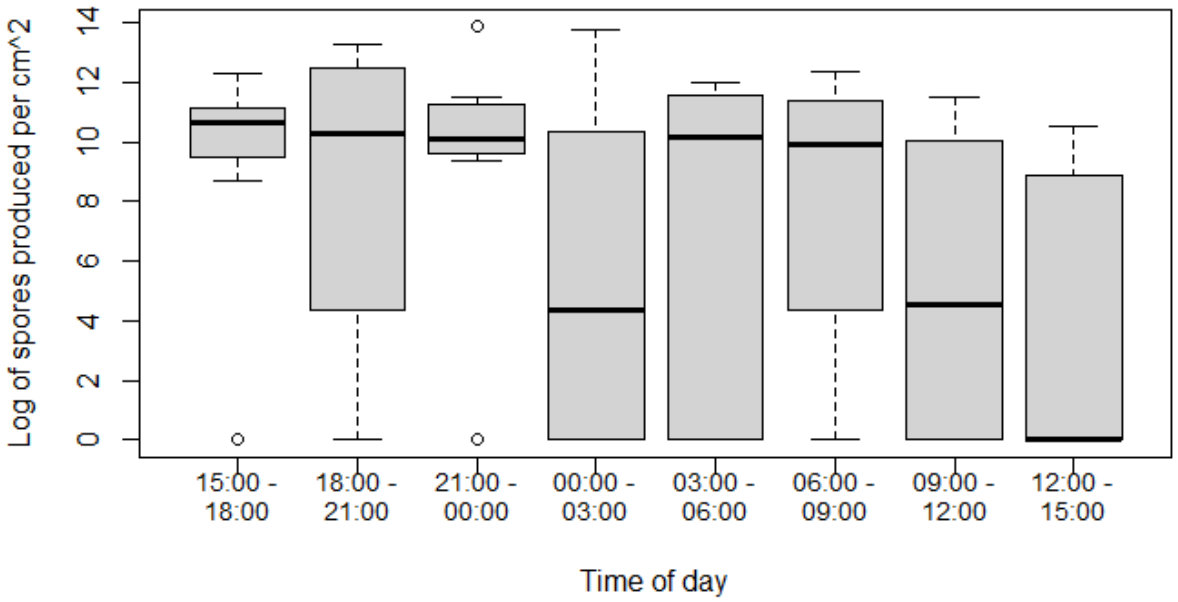


Figure 5. Boxplot of spore production (per cm^2 per 3 hours, log10-transformed) in four *Fomitopsis pinicola*- fruiting bodies from Nordre Pollen nature reserve, Norway, at 20.05 and 13.06.

Table 2. Summary of Anova test showing the effect of time (May and June) on spore production (per cm^2 per 3 hours, log10-transformed) in four *Fomitopsis pinicola* fruiting bodies on spruce snags in Nordre Pollen nature reserve, Norway.

	Df	**Sum sq**	**F value**	**P-value**
Timeframe	7	$2.1*10^{11}$	0.749	0.632
Residuals	56	$2.2*10^{12}$		

Table 3. Summary of Anova test showing the effect of day (06:00-20:59) and night (21:00-05:59) (May and June) on spore production (per cm^2 per 3 hours, log10-transformed) in four *Fomitopsis pinicola*- fruiting bodies on spruce snags in Nordre Pollen nature reserve, Norway.

	Df	Sum sq	F value	P-value
Day or Night	1	$4.2*10^{10}$	1.097	0.299
Residuals	62	$2.4*10^{12}$		

Table 4. Summary of regression test showing the effect of temperature on spore production (per cm^2 per 3 hours, log10-transformed) in four *Fomitopsis pinicola* fruiting bodies on spruce snags in Nordre Pollen nature reserve, Norway. Only data from June was included.

	Df	Sum sq	F value	P-value
Temperature	1	$1.6*10^{10}$	1.569	0.220
Residuals	30	$3.0*10^{11}$		

Table 5. Summary of regression test showing the effect of air humidity on spore production (per cm^2 per 3 hours, log10-transformed) in four *Fomitopsis pinicola* fruiting bodies on spruce snags in Nordre Pollen nature reserve, Norway. Only data from June was included.

	Df	Sum sq	F value	P-value
Humidity	1	$3.5*10^{9}$	0.335	0.567
Residuals	30	$3.1*10^{11}$		

3.2 Camera and image quality

Most cameras remained operational during the entire study period. Two cameras stopped working during the middle of the study period, and a two had some dates missing, however 6/9 cameras showed no large deviancy (active for >95% of days during the study period) from continuous operating times (Table 6).

A total of 132 590 photos were taken by the nine cameras through the summer. 35% (46 405) were sorted out as low-quality photos using the python script (didn't pass the threshold VL value). This left 86 165 photos for annotation.

During annotation 37% (34 333, 25% of total photos) of the high-quality photos proved to be of too low quality for beetle identification (false positives), not filtered out by the script during the initial image handling. Furthermore, the level of photo quality varied greatly between sites (Figure 6). The total number of high-quality photos were higher at day than night (Table 7) and lowest during May and July (<50% high-quality photos) (Table 8).

Table 6. Overview of dates with photos and dates without photos (April-September, 2020) from cameras capturing the fruiting bodies of nine *Fomitopsis pinicola* every ten minutes at different sites in Nordre Pollen and Østmarka.

Site ID	Dates with photos	Dates without photos
POL5	28.04 – 21.07, 23.08 – 02.09	22.07 – 22.08
POL4	28.04 – 02.09	0
POL3	28.04 – 02.09	0
POL2	28.04 – 02.09	0
OST6	17.04 – 18.09	0
OST5	17.04– 30.04, 01.06 – 01.07	01.05 – 31.05, 02.07 – 18.09
OST4	17.04 – 09.15	16.09 – 18.09
OST3	17.04 – 17.06	18.06 – 18.09
OST2	17.04 – 20.04, 23.04 – 18.09	21.04 – 22.04

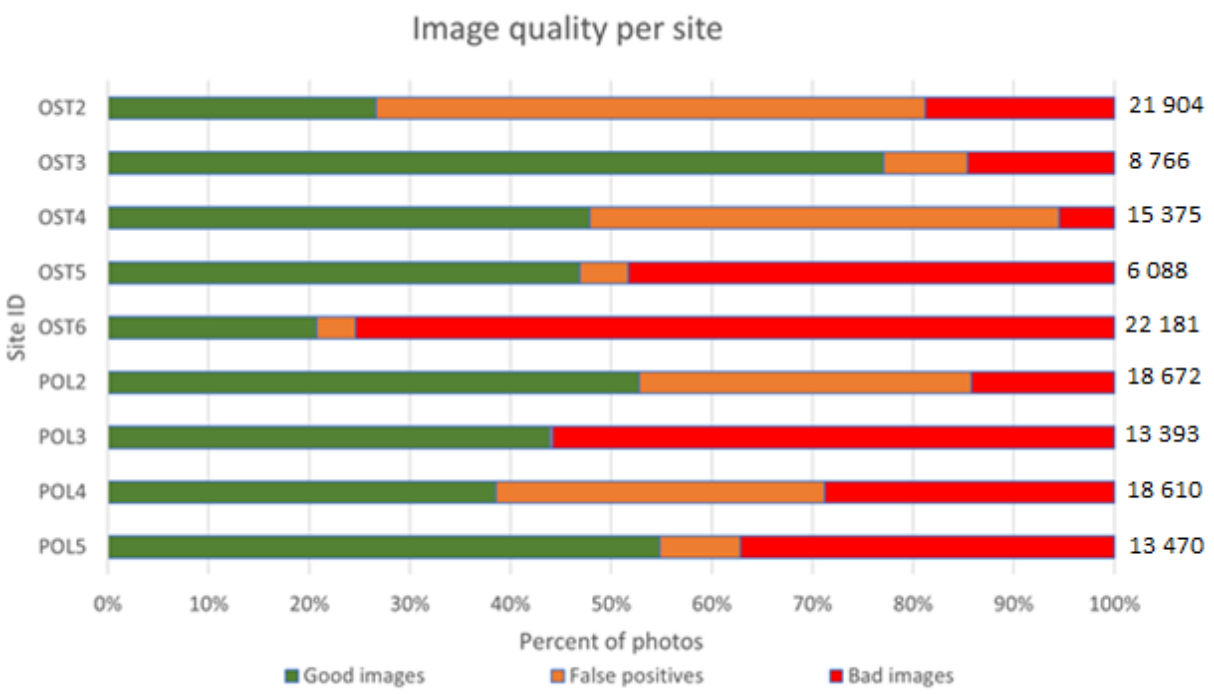


Figure 6. Photo quality, including falsely sorted high-quality images, in percent of total number of images per site. Total number of images is given to the right. Photos taken by cameras capturing the fruiting bodies of nine *Fomitopsis pinicola* every ten minutes at different sites in Nordre Pollen and Østmarka.

Table 7. Overview of high-quality photos taken at day vs. night (April-September, 2020) from cameras capturing the fruiting bodies of nine *Fomitopsis pinicola* every ten minutes at different sites in Nordre Pollen and Østmarka.

Locality	Site	Day	Night
Nordre Pollen	POL5	4178	3207
Nordre Pollen	POL4	5658	1522
Nordre Pollen	POL3	3370	2510
Nordre Pollen	POL2	6356	3504
Sum Nordre Pollen		19 562	10 743
Østmarka	OST6	1951	2651
Østmarka	OST5	1361	1495
Østmarka	OST4	5247	2118
Østmarka	OST3	4844	1914
Østmarka	OST2	2519	3316
Sum Østmarka		15 922	11 494
Total		35 484	22 237

Table 8. Overview of high-quality photos taken per month (April-September, 2020) from cameras capturing the fruiting bodies of nine *Fomitopsis pinicola* every ten minutes at different sites in Nordre Pollen and Østmarka.

	April	May	June	July	August	September
High-quality photos	6727	10075	17423	5664	10075	2317
Total photos	7052	20491	22731	19119	15801	3325
Percent	95%	49%	77%	30%	64%	70%

3.3 Beetle visits:

A total of 1851 beetles were observed on photos throughout the study period, and these were found on 2,7% of the total number of high-quality photos. An expert on beetles was able to identify eight species and one genus (Table 9).

Thymalus limbatus was the most frequently observed beetle overall, being observed throughout the whole summer in both forests. Second was *Peltis ferruginea* which only appeared at two polypores in Nordre Pollenvann, but with high numbers. *Ipidia binotata* and *Lordithon lunulatus* were both found primarily in Østmarka and showed a similar number of observations. The rest of the beetles identified were only observed 1-2 times throughout the season.

Table 9. Overview of number of beetle observations from 57721 photos. Images taken from cameras capturing the fruiting bodies of nine *Fomitopsis pinicola* every ten minutes, at different sites in Nordre Pollen and Østmarka

Species	Østmarka	Nordre Pollen	Total
Thymalus limbatus	927	254	1181
Peltis ferruginea	0	335	335
Ipidia binotata	85	11	96
Lordithon lunulatus	94	0	94
*Rhizophagus dispar**	1	1	2
*Arpidiphorus orbicularis***	0	1	1
Dendrophagus crenatus	1	0	1
Melanotus castanipes	1	0	1
Sepedophilus sp.	1	0	1
Unknown beetles	26	115	139
Total observations	1136	717	1851

* Correct genus, uncertain species

** Uncertain classification

The four beetle species with the most observations seemed to have different occurrences throughout the summer (Figure 7). *Ipidia binotata* was observed almost exclusively in May and July, while *Lordithon lunulatus* was almost exclusively observed during April. *Peltis ferruginea* had a large spike in observations during the middle of June, before rapidly dropping for the rest of the season. *Thymalus limbatus* was the only beetle that was observed across all months, with highest observations in June.

Patterns in observations throughout the day varied between species (Figure 8). One species, *T. limbatus,* was observed much more frequently during nighttime, while the other 3 species had more occurrences during daytime. Observations also seemed to vary with different temperatures, with higher temperatures giving more observations for most species (Figure 9).

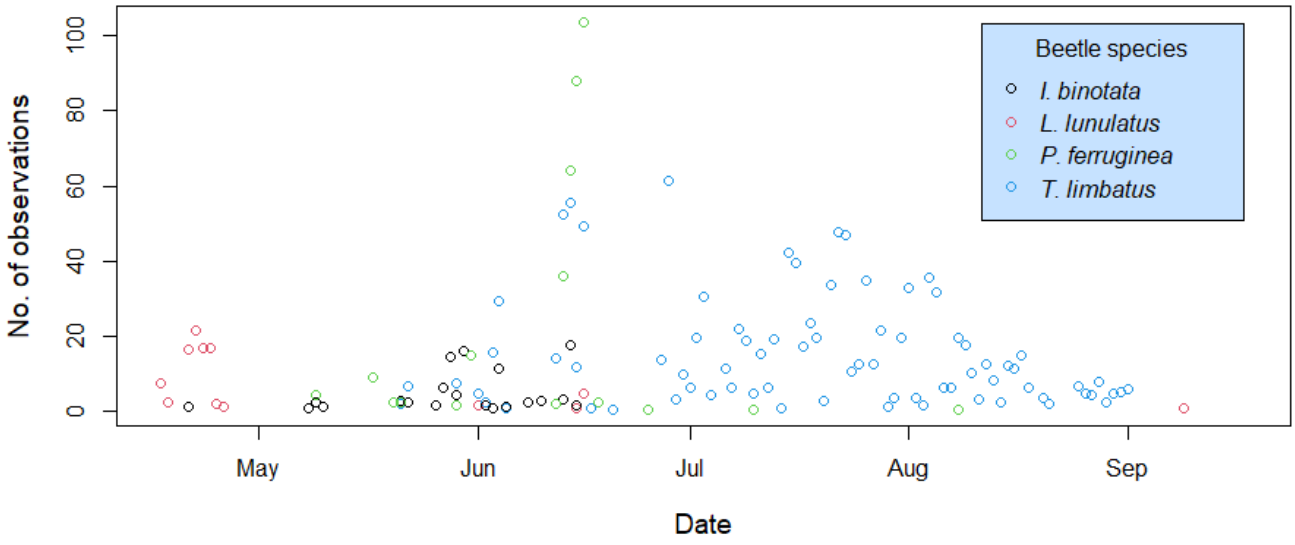


Figure 7. Observations of the beetle species *Ipidia binotata, Lordithon lunulatus, Peltis ferruginea and Thymalus limbatus* visiting nine fruit bodies of *Fomitopsis pinicola* in the period 17.04 to 18.09 in Østmarka and Nordre Pollen nature reserves at 1-day intervals, based on a total of 57721 photos taken at ten minute intervals.

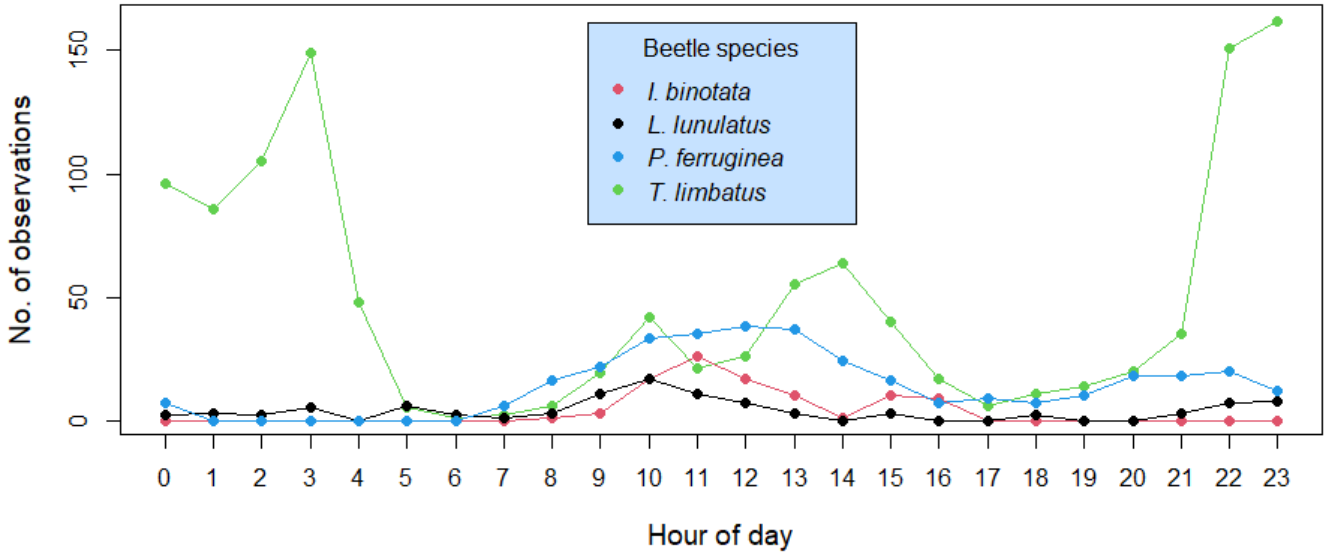


Figure 8. Observations of the beetle species *Ipidia binotata, Lordithon lunulatus, Peltis ferruginea and Thymalus limbatus* visiting nine fruit bodies of *Fomitopsis pinnicola* in the period 17.04 to 18.09 in Østmarka and Nordre Pollen nature reserves at 1-hour intervals, based on a total of 57721 photos taken at ten minute intervals.

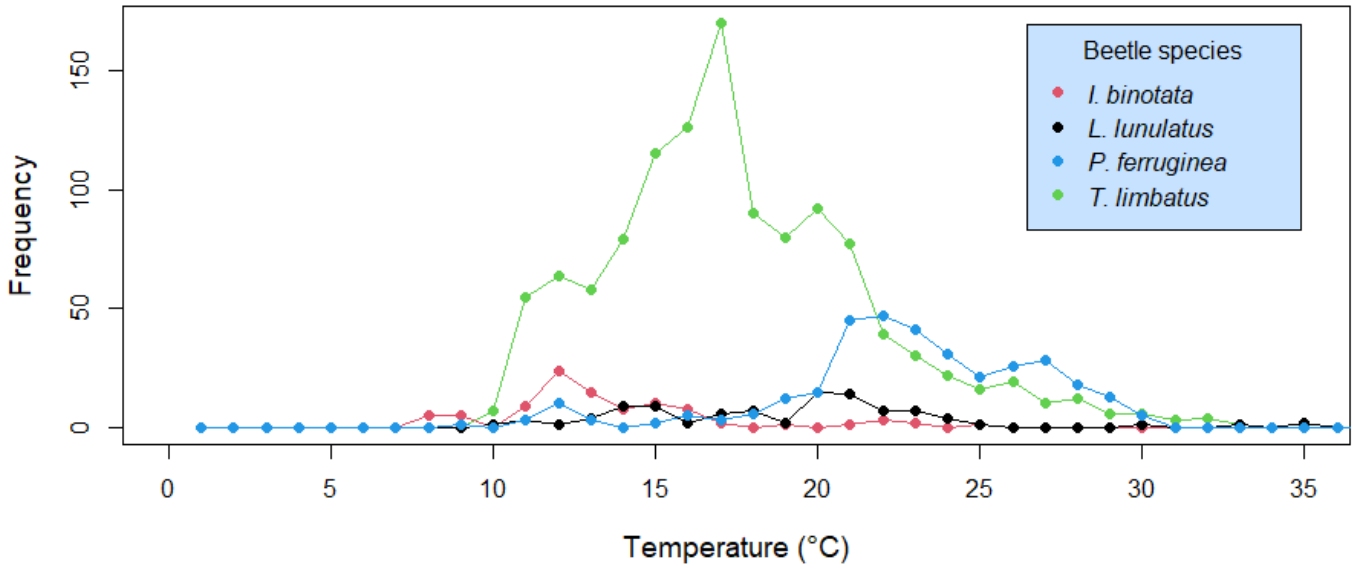


Figure 9. Observations of the beetle species *Ipidia binotata, Lordithon lunulatus, Peltis ferruginea and Thymalus limbatus* visiting nine fruit bodies of *Fomitopsis pinnicola* in the period 17.04 to 18.09 in Østmarka and Nordre Pollen nature reserves by 1 ºC-intervals, based on a total of 57721 photos taken at ten minute intervals.

3.4 Beetle visits in relation to spore production

Spore production could not explain frequency of beetle visits when looking at the combined data from May and June. However, the number of beetles increased with temperature and was at its highest during nighttime (Table 10).

When analyzing data from May separately, as this month represent the highest spore production, spore production was able explain the occurrence of beetles. The effect from hour of day and temperature was no longer present however (Table 11).

When the same analysis was conducted for July separately the effect of spore production disappeared, and temperature was the only explanatory variable of significance (Table 12).

Table 10. Results of GLMM analysis testing the effects spore production (log10-transformed), temperature and hour of day on beetles observed on *Fomitopsis pinicola* by photos taken at ten-minute intervals in Nordre Pollen nature reserve from 18.05.2020-24.05 & 08.06-13.06 using three-hour time intervals (Timeframe) and Site ID as random effects.

		Sum	
Predictors	Mean	CI	P-value
(Intercept)	-10.74	-14.97 – -6.51	**<0.001**
Spores produced	-0.03	-0.07 – 0.01	0.141
Temperature in ºC	0.19	0.12 – 0.26	**<0.001**
Hour of day	0.24	0.03 – 0.44	**0.024**

Random Effects:	Variance	Std.Dev.
Three-hour intervals	9.769	3.126
Site ID	2.832	1.683

Observations (N)	4445
Marginal R^2 / Conditional R^2	0.258 / 0.783

Table 11. Results of GLMM analysis testing the effects spore production (log10-transformed), temperature and hour of day on beetles observed on *Fomitopsis pinicola* by photos taken at ten-minute intervals in Nordre Pollen nature reserve from 18.05.2020-24.05 using three-hour time intervals (Timeframe) as a random effect.

		Sum	
Predictors	Log-Mean	CI	P-value
(Intercept)	-12.55	-16.21 – -8.89	**<0.001**
Spores produced	0.64	0.39 – 0.90	**<0.001**
Temperature in ºC	0.04	-0.10 – 0.18	0.564
Hour of day	-0.01	-0.16 – 0.15	0.949
Random Effects: *	Variance	Std.Dev.	
Three-hour intervals	1.742	1.32	
Observations (N)	2170		
Marginal R^2 / Conditional R^2	0.480 / 0.611		

*Site ID had a variance of 0 for this test and was therefore excluded

Table 12. Results of GLMM analysis testing the effects spore production (log10-transformed), temperature and hour of day on beetles observed on *Fomitopsis pinicola* by photos taken at ten-minute intervals in Nordre Pollen nature reserve from 08.06.2020-13.06 using three-hour time intervals (Timeframe) and Site ID as random effects.

		Sum	
Predictors	Mean	CI	P-value
(Intercept)	-11.34	-16.69 – -5.99	**<0.001**
Spores produced	0.00	-0.08 – 0.08	0.984
Temperature in ºC	0.21	0.10 – 0.32	**<0.001**
Hour of day	0.21	0.04 – 0.46	0.095

Random Effects:	Variance	Std.Dev.
Three-hour intervals	11.680	3.418
Site ID	3.081	1.755

Observations (N)	4445
Marginal R^2 / Conditional R^2	0.219 / 0.805

4 Discussion:

Contrary to expectations, I did not find higher spore production during nighttime, but the production varied noticeably throughout the season with highest numbers in May and June. Neither temperature, nor air humidity had effects on the spore production I measured. The number of beetles that visited the polypores increased with spores produced in May, but not in June. Our changes in analysis of photos and setup of cameras did not lead to significant improvements when compared to earlier years.

4.1 Variation in spore production

Spore production being significant between months, and at its highest in May, falls in line with what has previously been found on the sporulation patterns of *F. pinicola* (Nuss 1986, Hågvar 1999). Studies have also shown that time of day, temperature and humidity have impacts on spore production despite my study showing no effect from these factors (Haard and Kramer 1970, Hågvar 1999). Nuss (1986) found that spore production in *F. pinicola* needed a threshold value of around 0 ºC, below which, no sporulation would occur. There were no recorded temperatures below 0 ºC during my study period, which could explain why temperature did not appear to have any effect on spore production. Temperature values were similar both between months and through the day (only showing slightly cooler temperatures at night in May) in my study, despite large differences in sporulation between early and late months (Figure 4), which again can help explain why I did not observe a pattern. In addition, I had no temperature measurements from May, when Nuss (1986) observed the strongest temperature impacts (Nuss 1986). Humidity followed similar patterns to temperature in my study, in addition to also lacking measurements in May. This could explain why humidity, in a similar vein to temperature, showed no effect on spore production. Humidity has, however, been observed to be important for sporulation rate in other studies, due to spores being reliant on moisture to successfully germinate (Haard and Kramer 1970, Anco et al. 2013, Oneto et al. 2020). Many fungi have been observed to produce more spores during nighttime, following the levels of humidity, however little research has been done on this relationship for *F. pinicola* specifically. Hågvar (1999) observed occasional spikes in sporulation around midnight in the closely related species *F. fomentarius*, but this pattern was not observed in *F. pinicola* during my study. This could suggest that *F. pinicola* has different spore patterns, less reliant on time of day than other related polypores.

The discrepancies in my findings when compared to other studies, could however also be explained by the low number of days used for spore collecting. Using a single 24-hour period each month was likely not sufficient to properly quantify spore production throughout the day, especially given how only the first two months had spore numbers above 1 spore per sample. With May being the month with the highest spore production overall, it would be far more advantageous to focus on this month with multiple

measurements, preferably continuously throughout an entire week. It would also give stronger data to compare to the visits recorded from the time-lapse cameras, as single 24-hour intervals do not produce many beetle visits overall. If multiple measurements are to be performed per week it would likely require more than one person, as staying awake for more than 24 hours to gather samples is difficult for one individual.

4.2 Ecological information obtained from beetle visits

With *L. lunulatus, I. binotata and P. ferruginea* being mostly present during the early months of the season (Figure 7), it supports findings that they all primarily visit the hymenial layer to consume spores (Krasutskii 2007). In addition to consuming spores, *L. lunulatus* has also been observed to predate on other visiting beetles and has been classified as a generalist predator, therefore its frequency could also be affected by the visits of potential prey (Hågvar and Okland 1997, Fäldt et al. 1999, Krasutskii 2007). *T. limbatus* was more frequent through all months, which could be explained by it grazing on the hymenial layer of the polypore in addition to eating the spores it produces (Hågvar 1999). However, the high frequency of *T. limbatus* could potentially also be caused by its long visit times. Since each image with a beetle was counted as a separate visit for that species in order to reduce bias, there is the possibility that one individual was captured across multiple images, thus splitting up a single visit into multiple. *T. limbatus* has been shown to visit *F. pinicola* for as long as 32 hours, meaning up to 191 different visits could in reality be one singular (Ferkingstad 2020).

With time of day, the small increase in visits during the daytime (Figure 8), can be explained by daytime having approximately 60% more high-quality photos than nighttime (Table 7). The same problem appears for temperature, as it is a variable heavily correlated with time of day and therefore impacted by the discrepancy in photo quality between night and day. Looking at observations, the higher numbers of observed *T. limbatus* during the night seems to support it being more active at nighttime but can also be present during daytime. Ferkingstad (2020) found that time of day was the most important variable in explaining the activity of *T. limbatus*, stating that the

expected highest frequency of observations was at 1:11 a.m. (Ferkingstad 2020). *I. binotata* was only observed during the day, indicating its activity is largely day focused, however given only 96 total observations it suggests either scarcity or short visiting times, resulting in fewer individuals being captured on camera. Looking at comparisons between species we can see that *P. ferruginea* had a similar frequency to *T. limbatus* during the daytime, which could suggest that *P. ferruginea* is more active during the daytime. Alternatively it's possible that *T.limbatus* has comparatively low visiting numbers during the daytime, making *P.ferruginea* appear more frequent.

4.3 Ecological significance of relationship between beetle visits and spore production.

With May showing a positive relationship between spore production and beetle visits (Table 11), there is evidence to support that visiting frequency of fungivorous beetles are regulated by sporulation when production is high. Finding a relationship between beetles and their attraction to fruiting bodies of polypores falls in line with findings from multiple other studies (Jonsell and Nordlander 1995, Johansson et al. 2006). Johansson (2006) found that beetles can be drawn to volatiles emitted by wood infested with *F. pinicola*. While Jonsell and Nordlander (1995) found that odors emitted by the fungus during sporulation could attract beetles towards its fruiting body. Since the relationship was only positive during the month with the highest production, the visits seem to be driven by other factors when spore production is lower, mainly temperature and hour of day (Table 10). It should be noted that many of the observed species are active at different times throughout the summer, and have alternate diets when spores are less available (Fäldt et al. 1999, Hågvar 1999). Temperature, for instance, has been noted as important for species such as *P. ferruginea*, with colder temperatures generally being preferred for their distribution (Grammer 2018), which could explain its low frequency during the warm summer months in addition to spores being less prevalent (Figure 6).

The relationship between spore production and beetle visits from this study seems to enforce the idea that they play an important role in spore dispersal. While spore

dispersal by wind is largely random and has a limited range, insects dependent on dead wood are generally resource focused and can move with better precision from suitable substrate to another (Hågvar 1999, Komonen 2003, Vasiliauskas et al. 2005, Galante et al. 2011). Jacobsen (2018) found that limiting the access of invertebrates had significant effect on establishment of fungal communities in dead wood (Jacobsen et al. 2018b). Based on my findings the beetles most likely to act as spore dispersers in this study were *T. limbatus* and *P. ferruginea*, who both were frequent visitors during the predicted peak of spore production in late May and early July (Figure 7) (Nuss 1986). While no study has yet shown them to be attracted to volatiles emitted from *F. pinicola* during sporulation, they made up most visits used to generate the significant relationship between spore production and beetle visits in May. They have been shown to be active flyers during the sporulation period, making them ideal for spore dispersal from one suitable substrate to another (Gillespie et al. 2017). *T. limbatus* is an especially strong candidate given its high numbers and recently spores of *F. pinicola* have been shown to survive within the digestive tract of *T. limbatus* and successfully germinate afterwards (Lunde et al. [unpublished]).

4.4 Improvements on cameras & image quality

Overall a larger number of photos were taken this year (132 590), when compared to last year (84 354) (Ferkingstad 2020). This 57% increase in photos was partly attributed to a lengthier study period overall, starting a month earlier, and ending around the same time. More importantly Ferkingstad (2020) had significant downtime in many of his cameras during most of July, due to technical problems. This gave me an additional month of photos despite my study having two less cameras operating (Nine as opposed to 11). Longer operating times of my cameras also proves that the increased number of maintenance trips had good effect, especially given that all the cameras needed a battery change during the season.

With 40% (51 852) of total photos being good enough to identify visiting beetles, I saw an increase of 4% in high-quality photos compared to last year. While not a large

increase, it could be the result of some of the improvements made since the last study. One of the measures used to improve photo quality was implementing a new rubber seal between the camera lens and a glass that helped protect the lens, to help keep out moisture from away. Last years study used a foam seal instead of a rubber one leading to moisture being one of the main causes for low-quality images (Ferkingstad 2020). However, moisture ended up being one of the primary problems this year as well, so it is unlikely that this new rubber seal ensured less moisture. The issue reduced photo quality the most during the night, when the strong flash reflected of the water in the lens and made the photo too bright. This was also a problem without moisture, as the flash would occasionally light up the white hymenial layer of the polypore to such an extent that visiting arthropods would no longer be visible. The flash could potentially also impact beetle behavior, as sudden, bright lights have been proven to be disorienting to beetles active during the night (Horridge et al. 1983). Part of the issue with the rubber seal seemed to be how it was slightly too small to fit over the lens, so it had to be balanced on top and sealed with a plexiglass cover mounted above the seal. This cover was only fastened with one screw, leading to the glass being mounted in a slight skew. This probably allowed some moisture to enter from the other side, which then had trouble getting out thanks to the seal. A potential fix would either be a larger rubber seal, or a screw on the other side of the glass, to press the cover down better.

Despite more high-quality photos being taken during the day, there were still some problems that reduced the photo quality during this time. The main issue was glares from the sun, as all sites experienced some direct sunlight throughout daytime. Given the cameras being mounted at close to a 90º angle, the sunlight could reflect of the plastic covering the camera lens, making the photos unclear. While last years study listed this as a problem as well, similar studies using camera trapping earlier has not listed sun glare as an issue (Nichols et al. 2017, Hoye et al. 2021). This is likely because none of these studies had cameras mounted facing upwards. One possible solution to this issue would be to seek out polypores in more shaded areas but given the strict criteria already in place for selecting suitable polypores, further restricting available individuals might not be feasible. Alternatively, a polarizing filter could be applied over the camera lens, to help reduce the effect of sunlight in general.

Using the script calculating VL to filter out low-quality images led to worse results when compared to the study last year. With 20% (10 867) of photos being false positives last year compared to 25% (34 333) this year, we saw a 5% increase in false positives. This does seem to indicate that making a separate VL value for each site, instead of using one for all sites had a negative effect on reducing the number of false positives. The most likely explanation for this is that the number of photos used to calculate the VL value was too low per site. As the cameras were operating for a long time, the angle at which they captured images shifted slightly for some sites, most likely due to the weight of the cameras. This means there were high variance in what would be defined as a high-quality photo, something which could be solved by increasing the overall number of photos. It is important to make sure that the number of false positives is as low as possible, as they must be filtered out through manual annotation work, which is very time consuming and inefficient. For the future I believe that doubling the sample size from 100 to 200 could have better effects on the number of false positives.

Conclusions:

Use of cameras to monitor insect visits in polypores worked, but there is still room for improvement. Beetles visit polypores to consume spores, but it appeared that the visits only showed this interaction when spore production was at its highest. In order to ensure that this is a general pattern, new studies with more data are necessary. It was unexpected not finding increases in spore production during nighttime as well as finding high visiting numbers during daytime along with nighttime, if we exclude the most common species *T. limbatus*. This contrasts with earlier studies and needs to be repeated to be sure of the outcome. The fact that nine different beetle species was found on the nine randomly selected polypores does however imply that they are both an important food source for the insects and that spore dispersal is a likely outcome of the visits. This should therefore be researched further to answer if this is of ecological significance.

Anco, D. J., L. V. Madden, and M. A. Ellis. 2013. Effects of Temperature and Wetness Duration on the Sporulation Rate of Phomopsis viticola on Infected Grape Canes. Plant Disease **97**:579-589.

Bjerge, K., J. B. Nielsen, M. V. Sepstrup, F. Helsing-Nielsen, and T. T. Hoye. 2021. An Automated Light Trap to Monitor Moths (Lepidoptera) Using Computer Vision-Based Tracking and Deep Learning. Sensors **21**:18.

Boddy, L., and S. C. Watkinson. 1995. WOOD DECOMPOSITION, HIGHER FUNGI, AND THEIR ROLE IN NUTRIENT REDISTRIBUTION. Canadian Journal of Botany-Revue Canadienne De Botanique **73**:S1377-S1383.

Fäldt, J., M. Jonsell, G. Nordlander, and A.-K. Borg-Karlson. 1999. Volatiles of bracket fungi Fomitopsis pinicola and Fomes fomentarius and their functions as insect attractants. Journal of chemical ecology **25**:567-590.

Ferkingstad, B. J. 2020. Use of time-lapse cameras to monitor beetle activity on fruiting bodies of Fomitopsis pinicola. Norwegian University of Life Sciences.

Fourtouni, A., Y. Manetas, and C. Christias. 1998. Effects of UV-B radiation on growth, pigmentation, and spore production in the phytopathogenic fungus Alternaria solani. Canadian Journal of Botany-Revue Canadienne De Botanique **76**:2093-2099.

Galante, T. E., T. R. Horton, and D. P. Swaney. 2011. 95% of basidiospores fall within 1 m of the cap: a field-and modeling-based study. Mycologia **103**:1175-1183.

Gillespie, M. A., T. Birkemoe, and A. Sverdrup-Thygeson. 2017. Interactions between body size, abundance, seasonality, and phenology in forest beetles. Ecology and evolution **7**:1091-1100.

Grammer, J. B. 2018. Fund von Peltis ferruginea L., 1758 in Einödsbach, dem südlichsten Ort Deutschlands.

Guevara, R., A. D. M. Rayner, and S. E. Reynolds. 2000. Effects of fungivory by two specialist ciid beetles (Octotemnus glabriculus and Cis boleti) on the reproductive fitness of their host fungus, Coriolus versicolor. New Phytologist **145**:137-144.

Haard, R., and C. Kramer. 1970. Periodicity of spore discharge in the Hymenomycetes. Mycologia **62**:1145-1169.

Hågvar, S. 1999. Saproxylic beetles visiting living sporocarps of Fomitopsis pinicola and Fomes fomentarius. Norwegian Journal of Entomology **46**:25-32.

Hågvar, S. 2018. Contribution to the ecology of Gyrophaena boleti (Linnaeus, 1758)(Coleoptera, Staphylinidae) breeding in the pore layer of the fungus Fomitopsis pinicola (Fr.) Karst. Journal of Entomology **65**:108-114.

Hågvar, S., and B. S. B. Okland, Norwegian journal of entomology. 1997. SAPROXYLIC BEETLE FAUNA ASSOCIATED WITH LIVING SPOROCARPS OF FOMITOPSIS PINICOLA (FR.) KARST. IN FOUR SPRUCE FORESTS WITH DIFFERENT MANAGEMENT HISTORI ES. Fauna Norvegica **44**:95-105.

Heilmann-Clausen, J., and M. Christensen. 2004. Does size matter? On the importance of various dead wood fractions for fungal diversity in Danish beech forests. Forest Ecology and Management **201**:105-119.

Henderson Jr, C. R. 1982. Analysis of covariance in the mixed model: higher-level, nonhomogeneous, and random regressions. Biometrics:623-640.

Hongve, D. 1999. Production of dissolved organic carbon in forested catchments. Journal of Hydrology **224**:91-99.

Horridge, G. A., L. Marčelja, R. Jahnke, and P. McIntyre. 1983. Daily changes in the compound eye of a beetle (Macrogyrus). Proceedings of the Royal Society of London. Series B. Biological Sciences **217**:265-285.

Hoye, T. T., J. Arje, K. Bjerge, O. L. P. Hansen, A. Iosifidis, F. Leese, H. M. R. Mann, K. Meissner, C. Melvad, and J. Raitoharju. 2021. Deep learning and computer vision will transform entomology. Proceedings of the National Academy of Sciences of the United States of America **118**:10.

Jacobsen, R. M., H. Kauserud, A. Sverdrup-Thygeson, M. M. Bjorbwkmo, and T. Birkemoe. 2017. Wood-inhabiting insects can function as targeted vectors for decomposer fungi. Fungal Ecology **29**:76-84.

Jacobsen, R. M., A. Sverdrup-Thygeson, H. Kauserud, and T. Birkemoe. 2018a. Revealing hidden insect–fungus interactions; moderately specialized, modular and anti-nested detritivore networks. Proceedings of the Royal Society B: Biological Sciences **285**:20172833.

Jacobsen, R. M., A. Sverdrup-Thygeson, H. Kauserud, S. Mundra, and T. Birkemoe. 2018b. Exclusion of invertebrates influences saprotrophic fungal community and wood decay rate in an experimental field study. Functional Ecology **32**:2571-2582.

Johansson, T., J. Olsson, J. Hjalten, B. G. Jonsson, and L. Ericson. 2006. Beetle attraction to sporocarps and wood infected with mycelia of decay fungi in old-growth spruce forests of northern Sweden. Forest Ecology and Management **237**:335-341.

Jonsell, M., and G. Nordlander. 1995. Field attraction of Coleoptera to odours of the wood-decaying polypores Fomitopsis pinicola and Fomes fomentarius. Pages 391-402 *in* Annales Zoologici Fennici. JSTOR.

Jonsson, B. G., N. Kruys, and T. Ranius. 2005. Ecology of species living on dead wood - Lessons for dead wood management. Silva Fennica **39**:289-309.

Kebli, H., P. Drouin, S. Brais, and G. Kernaghan. 2011. Species Composition of Saproxylic Fungal Communities on Decaying Logs in the Boreal Forest. Microbial Ecology **61**:898-910.

Komonen, A. 2003. Distribution and abundance of insect fungivores in the fruiting bodies of Fomitopsis pinicola. Annales Zoologici Fennici **40**:495-504.

Krasutskii, B. 2007. Coleoptera associated with Fomitopsis pinicola (Sw.: Fr.) Karst.(Basidiomycetes, Aphyllophorales) in the forests of the Urals and Transurals. Entomological Review **87**:848-858.

Miljødirektoratet. 2002. Østmarka naturreservat. Miljødirektoratet, https://faktaark.naturbase.no/?id=VV00001859.

Miljødirektoratet. 2005. Pollen naturreservat. Miljødirektoratet, https://faktaark.naturbase.no/?id=VV00000850.

Muller, M. M., M. Varama, J. Heinonen, and A. M. Hallaksela. 2002. Influence of insects on the diversity of fungi in decaying spruce wood in managed and natural forests. Forest Ecology and Management **166**:165-181.

Nichols, M., A. S. Glen, P. Garvey, and J. Ross. 2017. A comparison of horizontal versus vertical camera placement to detect feral cats and mustelids. New Zealand Journal of Ecology **41**:145-150.

Norden, B., M. Ryberg, F. Gotmark, and B. Olausson. 2004. Relative importance of coarse and fine woody debris for the diversity of wood-inhabiting fungi in temperate broadleaf forests. Biological Conservation **117**:1-10.

Norros, V., R. Penttila, M. Suominen, and O. Ovaskainen. 2012. Dispersal may limit the occurrence of specialist wood decay fungi already at small spatial scales. Oikos **121**:961-974.

Nuss, I. 1986. Zur Ökologie der Porlinge II.

Oneto, D. L., J. Golan, A. Mazzino, A. Pringle, and A. Seminara. 2020. Timing of fungal spore release dictates survival during atmospheric transport. Proceedings of the National Academy of Sciences of the United States of America **117**:5134-5143.

Patriquin, M. N., J. R. Parkins, and R. C. Stedman. 2007. Socio-economic status of boreal communities in Canada. Forestry **80**:279-291.

Schigel, D. S. 2012. Fungivory and host associations of Coleoptera: a bibliography and review of research approaches. Mycology **3**:258-272.

Steen, R., and M. Barmoen. 2017. Diel activity of foraging Eurasian red squirrels (Sciurus vulgaris) in the winter revealed by camera traps. Hystrix-Italian Journal of Mammalogy **28**:43-47.

Talbot, J. M., S. D. Allison, and K. K. Treseder. 2008. Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. Functional Ecology **22**:955-963.

Ulyshen, M. D. 2016. Wood decomposition as influenced by invertebrates. Biological Reviews **91**:70-85.

Vasiliauskas, R., V. Lygis, K.-H. Larsson, and J. Stenlid. 2005. Airborne fungal colonisation of coarse woody debris in North Temperate Picea abies forest: impact of season and local spatial scale. Mycological research **109**:487-496.

Weslien, J., L. B. Djupstrom, M. Schroeder, and O. Widenfalk. 2011. Long-term priority effects among insects and fungi colonizing decaying wood. Journal of Animal Ecology **80**:1155-1162.

Zuur, A., E. N. Ieno, N. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed effects models and extensions in ecology with R. Springer Science & Business Media.

Appendix 1: Python script for calculating the Variance of Laplacian (VL) in photos

```
# -*- coding: utf-8 -*-
"""
Created on Tue Oct 22 16:51:02 2019

This script calculates the variance of the Laplacian (VL) as a measure for sharpness of an image for each image in a given directory.
The mean and SD is calculated.

The SortImages function sorts images in a given directory on the basis of a threshold value.

@author: Hjalte Mann

"""

##############
### Import the necessary packages

import cv2 # Handling images. This is the openCV package and it needs to be installed.
from statistics import mean, pstdev # Calculate mean and SD
import os #Create folders etc
import shutil # Move files
```

```
###
##############



##############
### Set paths til the relevant folders
path_to_good = "D:/Example/Good" # Folder with good images (manually categorised)
path_to_test = "D:/Example/Test" # Folder with images that need to be sorted
###
##############



##############
### Make a couple of empty lists
blur_good = [] # Make an empty list that will contain the VL's for the good images
blur_bad = [] # Make an empty list that will contain the VL's for the bad images
###
##############



##############
### Below three different functions are defined.


def variance_of_laplacian(image):
```

```
    # Function that computes the Laplacian of the image and then returns the focus
    # measure, which is simply the variance of the Laplacian
    return cv2.Laplacian(image, cv2.CV_64F).var()


def GetBlurry(path, output_list):
    # Function that loops over the images in a directory, calculates the VL and append the value to a list.
    # The function needs the path to the images (path) and the name of the list to output to (output_list).
    for images in os.listdir(path): # Loop over images in the given directory
        path_to_image = os.path.join(path, images) # Set the full path to an image
        image = cv2.imread(path_to_image) # Load the image
        gray = cv2.cvtColor(image, cv2.COLOR_BGR2GRAY) # Convert the image to grayscale
        VL = variance_of_laplacian(gray) # Calculate VL with the variance_of_laplacian-function
        output_list.append(VL) # Append the VL value to the output_list


def SortImages(path):
    # Function that sort images on the basis of their VL and a given threshold.
    sorted_good = os.path.join(path, "sorted_good") # Create a string with the path for sorted good images
    sorted_bad = os.path.join(path, "sorted_bad") # Create a string with the path for sorted bad images
```

os.mkdir(sorted_good) # Create the directory (folder) for the sorted_good images in the test directory

os.mkdir(sorted_bad) # Create the directory (folder) for the sorted_bad images in the test directory

for images in os.listdir(path): # Loop over images in the direcotry given by "path"

if images.endswith('.JPG'): # But only do the rest if the filename ends with .JPG (we have just created two folders in the test directory and we don't want to include these in the next.)

path_to_image = os.path.join(path, images) # Set the full path to the image

image = cv2.imread(path_to_image) # Load the image

gray = cv2.cvtColor(image, cv2.COLOR_BGR2GRAY) #Convert the image to grayscale

VL = variance_of_laplacian(gray) # Calculate VL with the variance_of_laplacian-function

if VL > threshold: # If the VL for the image is above the given threshold...

shutil.move(path_to_image, sorted_good) #... then move it to the sorted_good folder

if VL < threshold: # If the VL for the image is below the given threshold...

shutil.move(path_to_image, sorted_bad) #... then move it to the sorted_bad folder

###

##############

##############

Now we use our function on our data

GetBlurry(path_to_good, blur_good) # Run out GetBlurry-function on the good images. Set the output_list to blur_good (the empty list we defined earlier in the script)

#GetBlurry(path_to_bad, blur_bad)# Run out GetBlurry-function on the bad images. Set the output_list to blur_bad (the empty list we defined earlier in the script)

good_mean = mean(blur_good) # Calculate the mean VL for the good images

good_sd = pstdev(blur_good) # Calculate the VL SD for the good images

bad_mean = mean(blur_bad) # Calculate the mean VL for the bad images

bad_sd = pstdev(blur_bad) # Calculate the VL SD for the bad images

threshold = good_mean - good_sd # Set the threshold as VL-SD (values from good images)

SortImages(path_to_test) # Run our SortImages function on the images in the test folder. The function uses the threshold above, which can also just be set manually (e.g. threshold = 5)

###

##############

##############

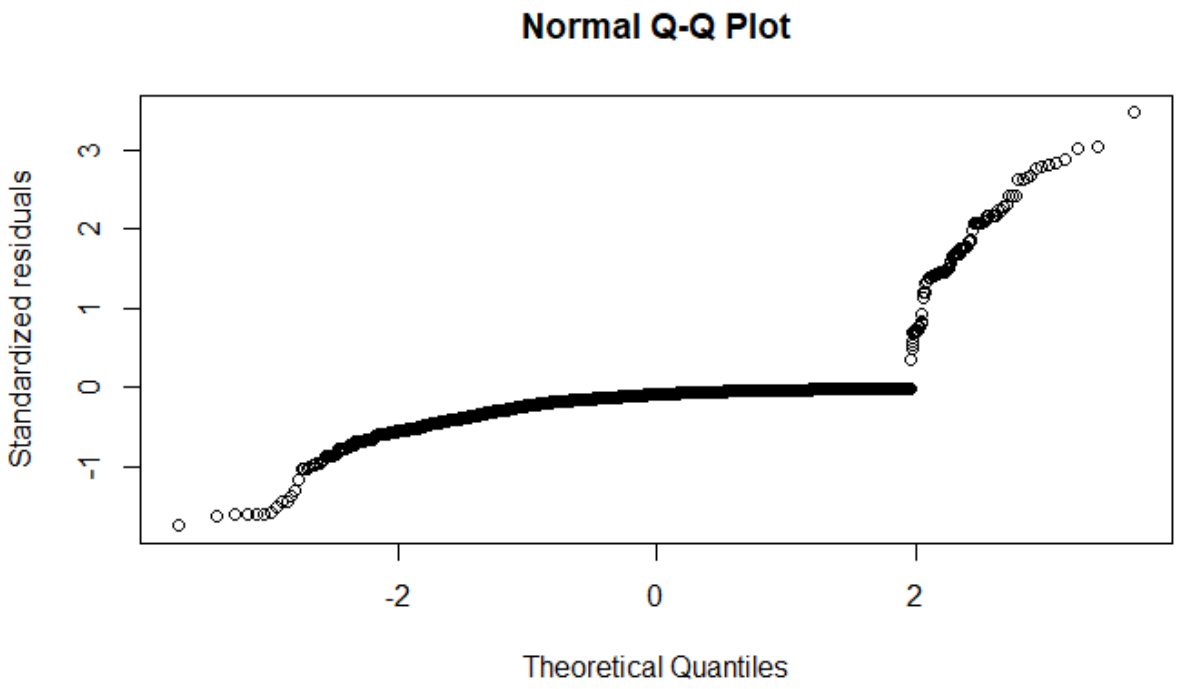
Print some stuff

print("Mean VL for good: ", good_mean)

```
print("VL SD for good: ", good_sd)

print("Mean VL for bad: ", bad_mean)

print("VL SD for bad: ", bad_sd)

print("Threshold set to: ", threshold)

print("All done")


###

##############
```

Appendix 2: Model diagnostics from the General Linear Mixed Models

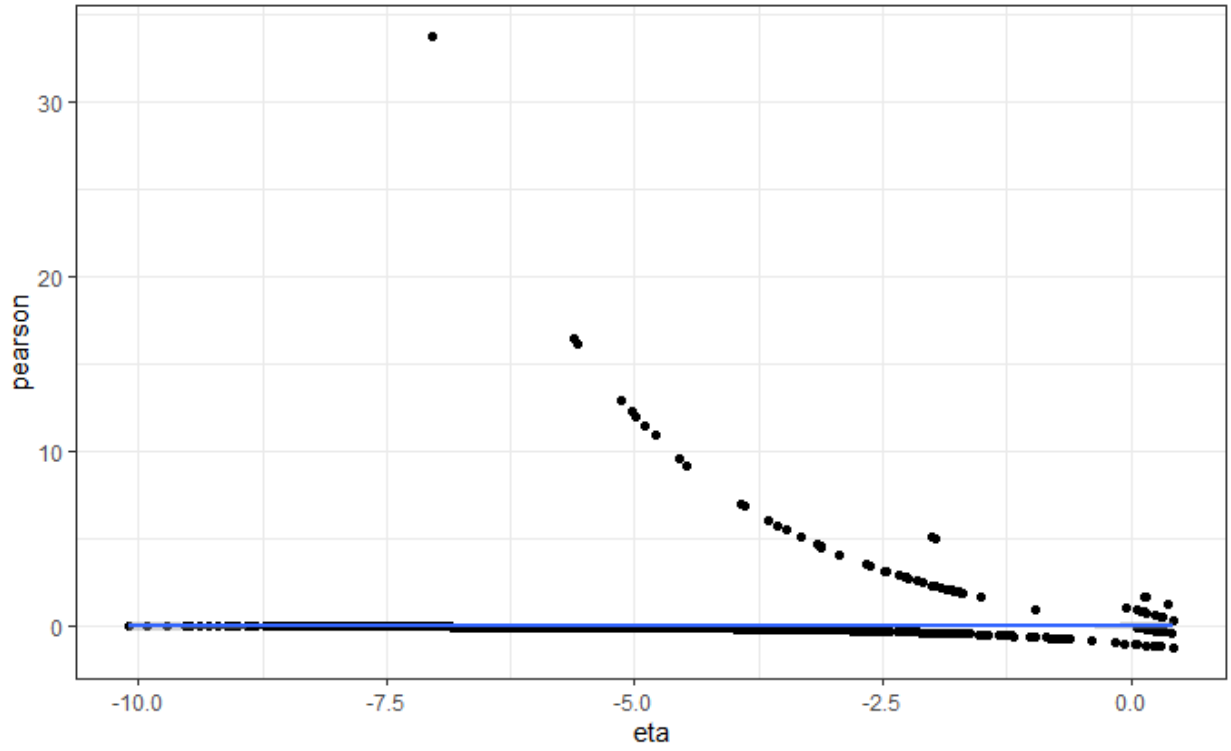
1 Analysis including both May and June:



Appendix 2.1. Q-Q plot of distribution of residuals for GLMM analysis testing the effects spore production (log10-transformed), temperature and hour of day on beetles observed on

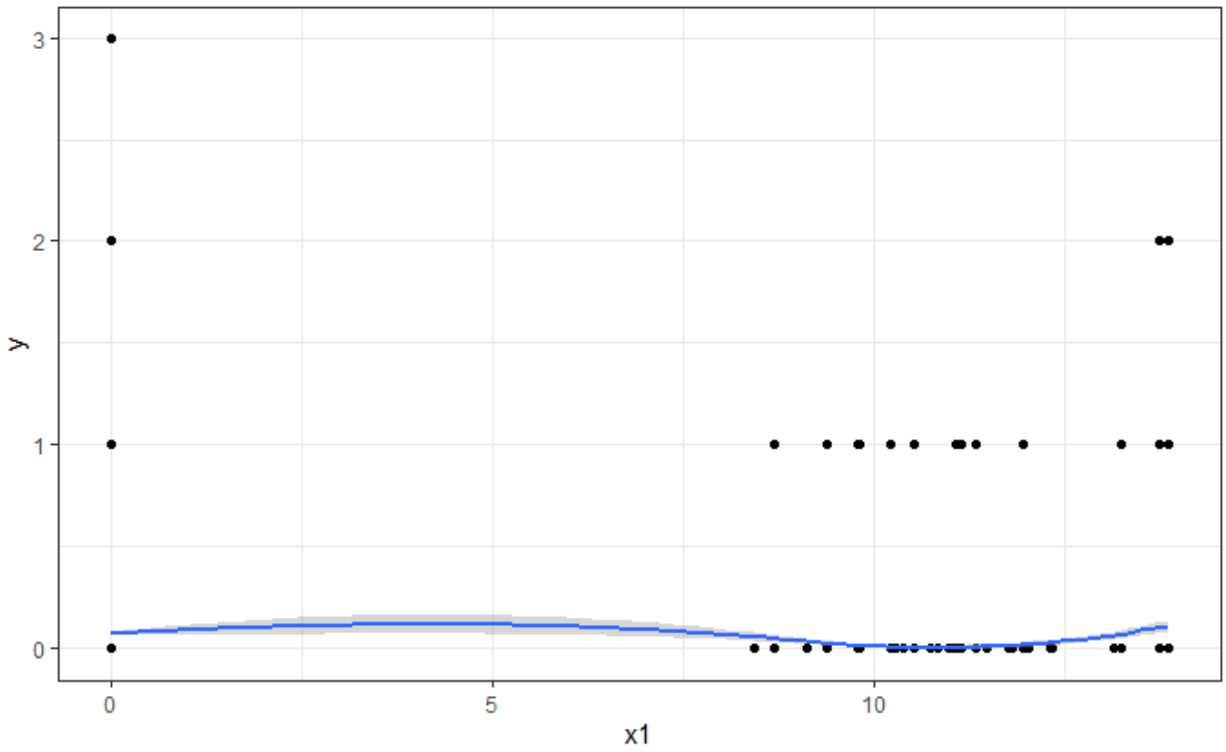
Fomitopsis pinicola by photos taken at ten-minute intervals in Nordre Pollen nature reserve from 18.05.2020-24.05 & 08.06-13.06 using three-hour time intervals (Timeframe) and Site ID as random effects.

There appears to be deviation from the normality of residuals assumption for the analysis from May and June.



Appendix 2.2. Plot of the residuals versus the response variable for GLMM analysis testing the effects spore production (log10-transformed), temperature and hour of day on beetles observed on *Fomitopsis pinicola* by photos taken at ten-minute intervals in Nordre Pollen nature reserve from 18.05.2020-24.05 & 08.06-13.06 using three-hour time intervals (Timeframe) and Site ID as random effects.

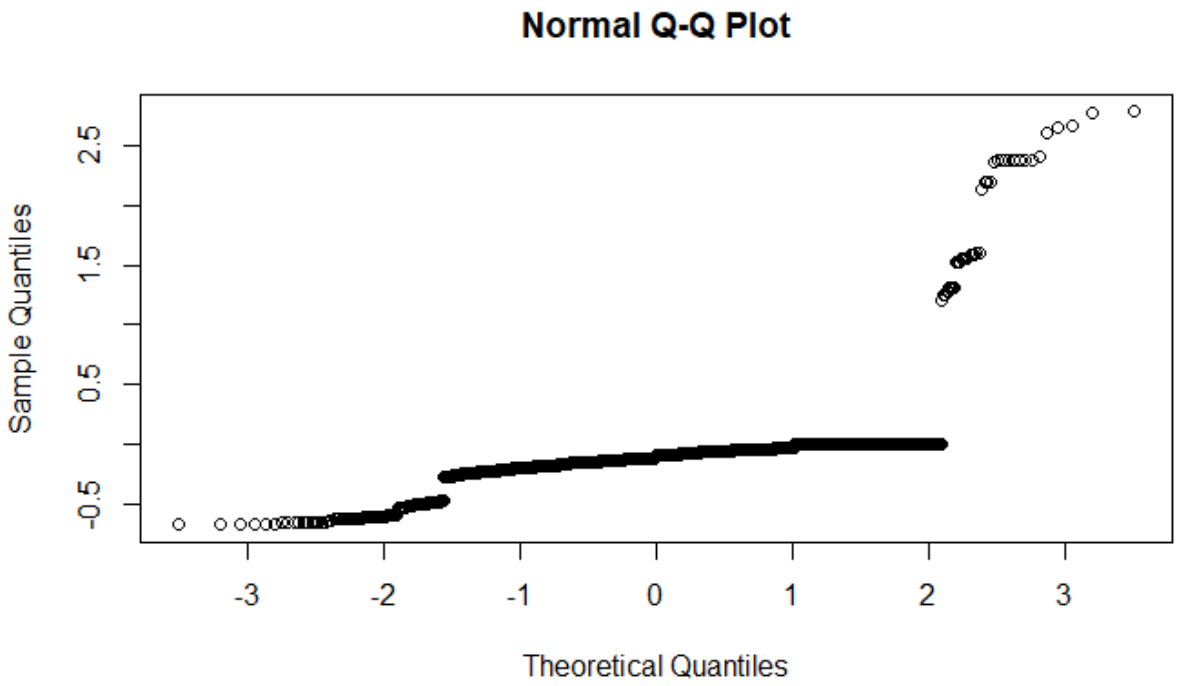
Response variable looks linear in accordance with the residuals in the fitted model for the analysis from May and June.



Appendix 2.3. Plot of the number of beetle visits versus spore production (log10-transformed) variables used in GLMM analysis testing the effects spore production (log10-transformed), temperature and hour of day on beetles observed on *Fomitopsis pinicola* by photos taken at ten-minute intervals in Nordre Pollen nature reserve from 18.05.2020-24.05 & 08.06-13.06 using three-hour time intervals (Timeframe) and Site ID as random effects.

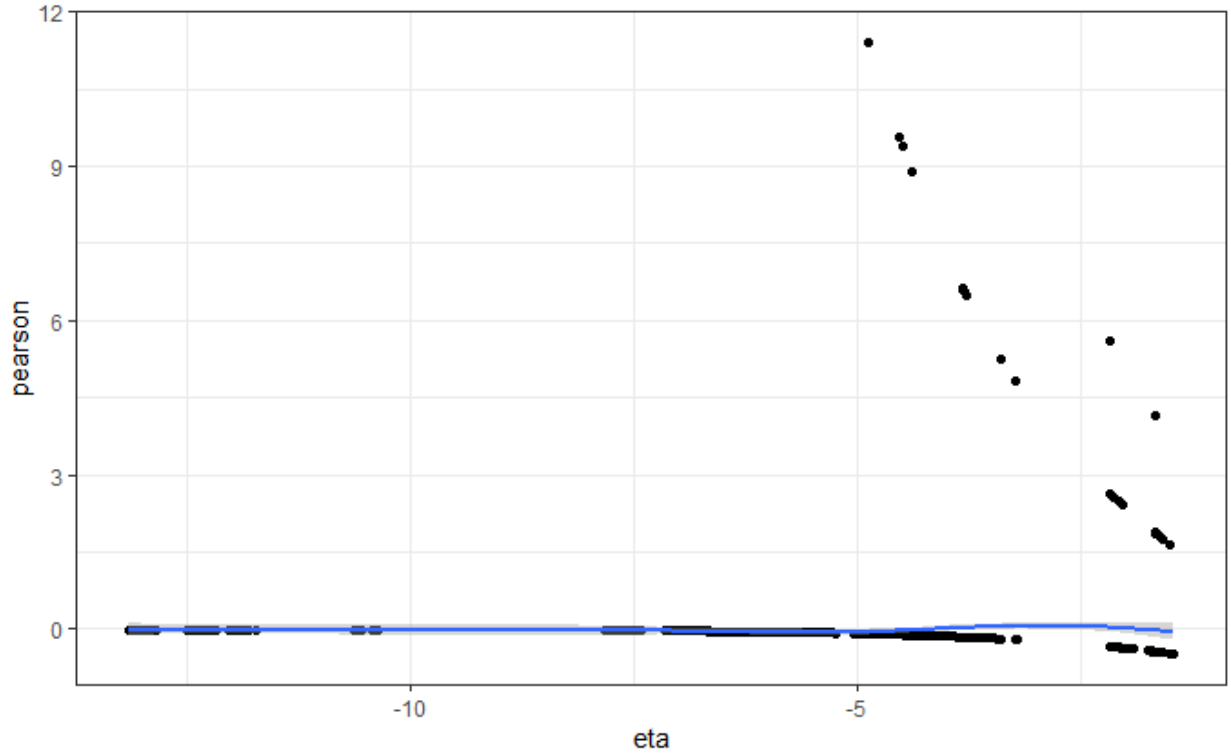
Assumption of Poisson appears to be violated, due to no linearity in spores variable for the analysis from May and June.

2 Analysis from May



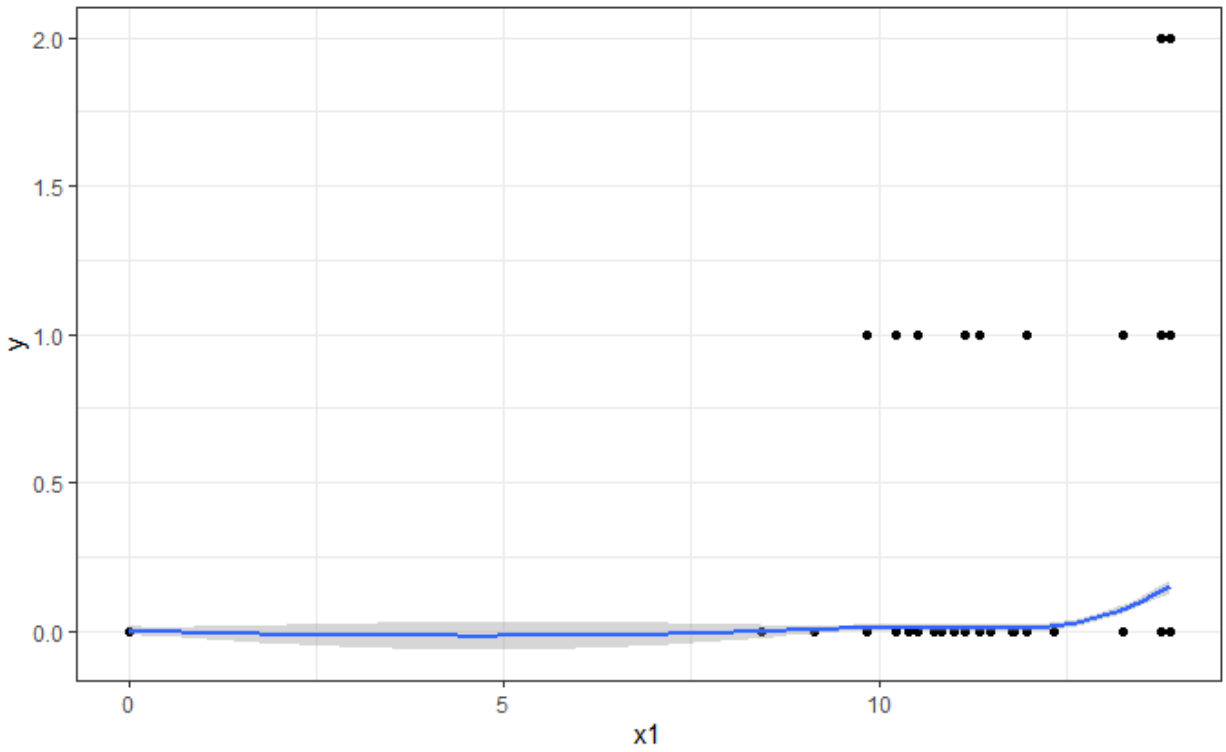
Appendix 2.4. Q-Q plot of distribution of residuals for GLMM analysis testing the effects spore production (log10-transformed), temperature and hour of day on beetles observed on *Fomitopsis pinicola* by photos taken at ten-minute intervals in Nordre Pollen nature reserve from 18.05.2020-24.05 using three-hour time intervals (Timeframe) and Site ID as random effects.

There appears to be deviation from the normality of residuals assumption for the analysis from May.



Appendix 2.5. Plot of the residuals versus the response variable for GLMM analysis testing the effects spore production (log10-transformed), temperature and hour of day on beetles observed on *Fomitopsis pinicola* by photos taken at ten-minute intervals in Nordre Pollen nature reserve from 18.05.2020-24.05 using three-hour time intervals (Timeframe) and Site ID as random effects.

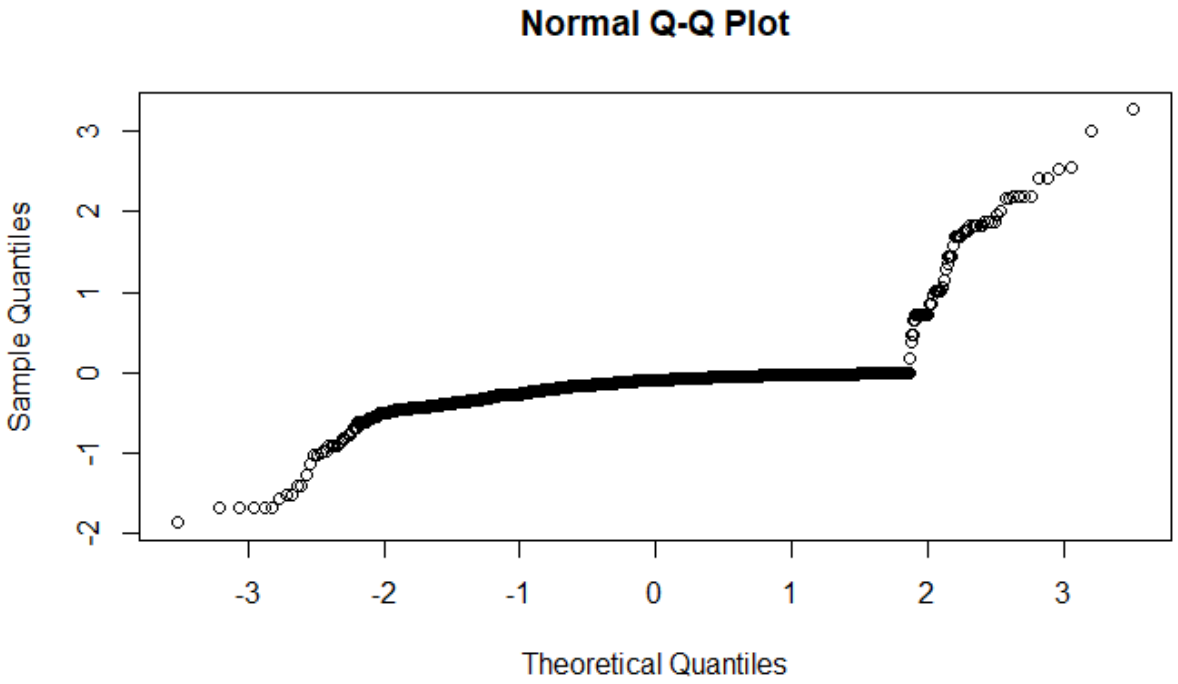
Response variable looks linear in accordance with the residuals in the fitted model for the analysis from May.



Appendix 2.6. Plot of the number of beetle visits versus spore production (log10-transformed) variables used in GLMM analysis testing the effects spore production (log10-transformed), temperature and hour of day on beetles observed on *Fomitopsis pinicola* by photos taken at ten-minute intervals in Nordre Pollen nature reserve from 18.05.2020-24.05 using three-hour time intervals (Timeframe) and Site ID as random effects.

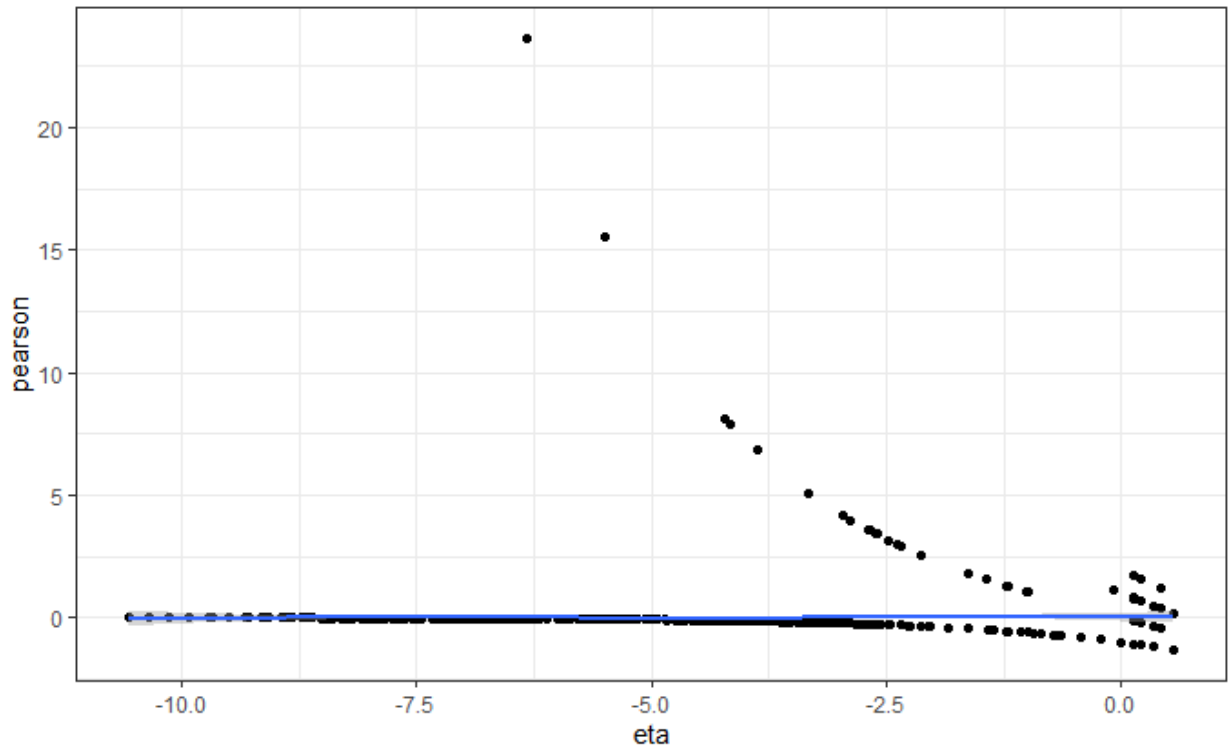
Assumption of Poisson appears to be violated, due to no linearity in spores variable for the analysis from May.

3 Analysis from June



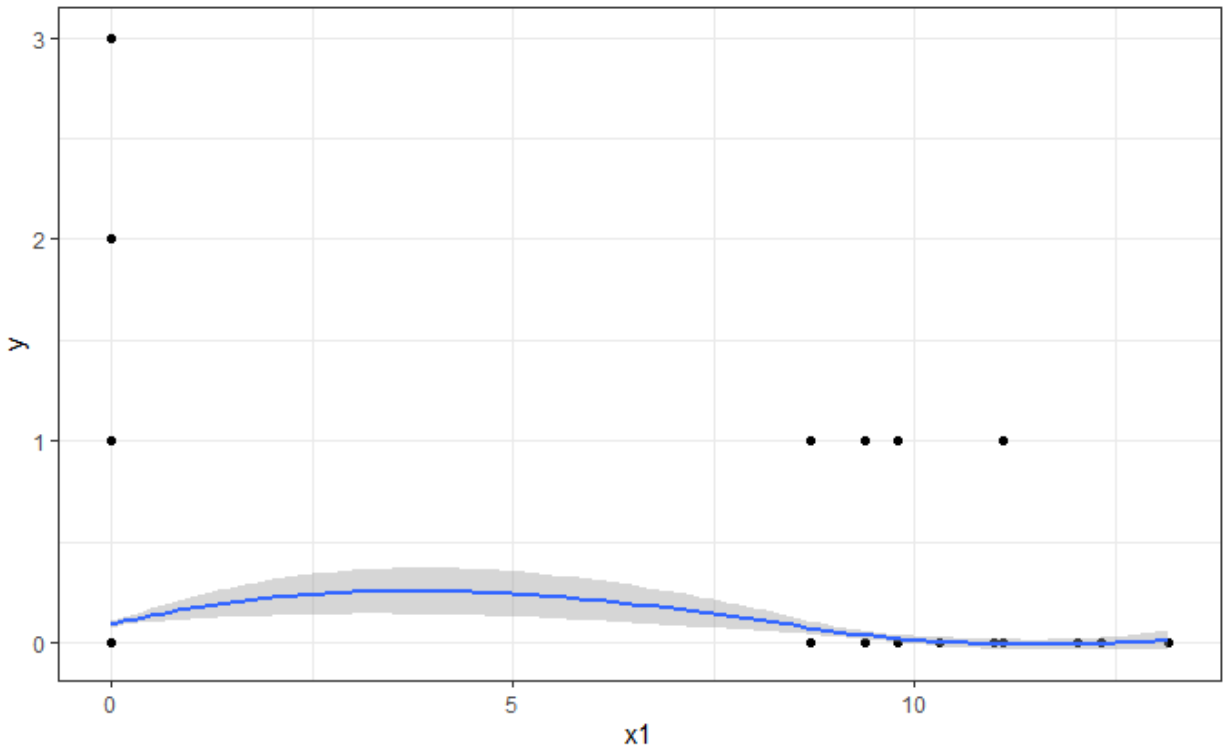
Appendix 2.7. Q-Q plot of distribution of residuals for GLMM analysis testing the effects spore production (log10-transformed), temperature and hour of day on beetles observed on *Fomitopsis pinicola* by photos taken at ten-minute intervals in Nordre Pollen nature reserve from 08.06-13.06 using three-hour time intervals (Timeframe) and Site ID as random effects.

There appears to be deviation from the normality of residuals assumption for the analysis from June.



Appendix 2.8. Plot of the residuals versus the response variable for GLMM analysis testing the effects spore production (log10-transformed), temperature and hour of day on beetles observed on *Fomitopsis pinicola* by photos taken at ten-minute intervals in Nordre Pollen nature reserve from 08.06-13.06 using three-hour time intervals (Timeframe) and Site ID as random effects.

Response variable looks linear in accordance with the residuals in the fitted model for the analysis from June.



Appendix 2.9. Plot of the number of beetle visits versus spore production (log10-transformed) variables used in GLMM analysis testing the effects spore production (log10-transformed), temperature and hour of day on beetles observed on *Fomitopsis pinicola* by photos taken at ten-minute intervals in Nordre Pollen nature reserve from 18.05.2020-24.05 & 08.06-13.06 using three-hour time intervals (Timeframe) and Site ID as random effects.

Assumption of Poisson appears to be violated, due to no linearity in spores variable for the analysis from May and June.

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