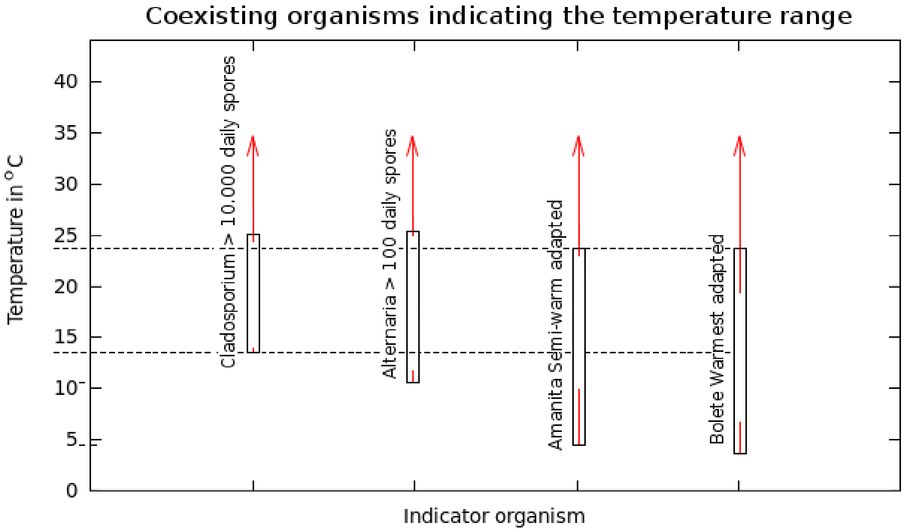
Danish Climate Centre Report

How to calibrate bio-indicators and assess changes in Climate and in Biodiversity



Title:
How to calibrate bio-indicators and assess changes in Climate and in Biodiversity.

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When do temperature and precipitation reflect phenological changes?

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Dansk resumé

Baggrund: En organisme kan anvendes til at indikere dens 'opfattede' nærmiljø, hvis den afspejler en definerbar tolerance frem for ukendte faktorer. Organismer, der således klart indikerer en veldefineret klimaparameter anses som en kalibreret indikator – en bio-proxy. Idet man efterhånden finder flere bio-proxies, der hver indikerer et afgrænset interval, vil sam-eksistens betyde, at de kun findes her, når deres fælles klimatolerance er tilgodeset (forside-figuren). Mange steder er der f.eks. skiftende nedbør hver time eller dag. Derfor er det kun korttids-fænologiske stadier, såsom når frøkapsler sprænges, som reelt kan indikere organismernes omgivende klimatolerancer på det pågældende tidspunkt og sted. For at kunne indikere klima, skal man genfinde verificerede bio-indikatorer igennem årtier.
Nogle arter kan karakteriseres ift. deres klima-zone, hvilket kan vidne om at disse mulige klima-tilpassede arter afspejler deres klimaparametre.

Formål med denne screening-undersøgelse er at finde metoder til at opdage mulige bio-indikatorer, vel-fungerende procedurer til at kalibrere dem, og kvalificerede bud på klimapåvirkede organismer og processer, der er værd at undersøge nøjere. Eksempelvis vurderes her mulige indikatorer for, hvorvidt de eksploderer i antal ift. årstiderne fordi de sæsonafhængige, eller om de afspejler at alle afgørende vejrforhold netop er rigtige til at sprede sig. Skønt alle vekselvarme organismer er potentielle bio-proxies, er der fokuseret på 2 slægter af mikrosvampe, skimmelsvampene Alternaria og Cladosporium, samt 2 rodforøgende mycorrhiza macrosvampe - dvs. fluesvampene (Amanita) og rørhattene (Bolete fungi). Dvs i alt 39 arter, hvoraf 6 kan være anvendelige som bio-proxies. Det

er type-eksempler fra sektor-forskningen, der kan bidrage med grundlæggende naturvidenskabelige resultater og de berørte brancher: Under screeningen søges der efter en biologisk indikation af, hvordan nedbør og temperatur var på et præcis tidspunkt og sted 'in situ' - uanset om det er i for-historisk tid eller i dag. I samme arbejdsgang, afdækker screeningen, hvordan organismer og klima interagerer - grundlæggende for biodiversitet og naturplanlægning, og resultater for allergi-, skov- og landbrugs-brancherne. Der er fundet bud på at:
- bio-proxies findes blandt almindelige arter under høj sporespredning eller fra særlige klimazoner.
- allergene niveauer af skimmelsvampene spredes når temperaturen er over 11°C i 15ms højde,
- forøget nedbør ser ud til at være mindst ligeså vigtig for kold-tilpassede arter som opvarmning, hvorved artssammensætningen ændres. Der findes færre kulde- og nedbørsfølsomme mycorrhiza-arter frem for nedbørs-indifferente arter. Det er dårligt nyt for nåletræer, men godt for løvtræerne.
Fremadrettet ønskes disse bio-proxies anvendt til at verificere klimamodellerne, og for at indgå i Bioklimatiske kort fra før termometrets tid, nutid og til klimaprognoser. Vi får dermed løbende lokalindsigt i hvordan klimaet har spillet sammen med fortidens kulturer, hvornår klimatilpasninger er vigtigere end andet for miljøreguleringer og arealanvendelser, i tide finde skadevoldere og nye afgrøder, samt øge interessen for naturlige ressourcer og ændrede klima-vilkår.

Abstract

Especially poikilotherm organisms are directly affected by their tolerance to climate conditions. Indirectly, we all respond to climate through our usage of natural resources and our place in the food-chain. So it is likely that some of us mirror our climate due to our tolerances. Few pre-industrial data are available on a local and time specific basis without reliable bio-proxies. In this screening study, selected indicator-organisms are calibrated according to their tolerances and assessed for bio-proxy purposes:

To reconstruct in situ precipitation and temperature for a specific location and time, 6 out of 39 of the bio-indicators studied are found applicable as proxies. Examples are selected from cosmopolite microfungi and from climate-zone adapted macrofungi. That is the 2 mold microfungi gena, Alternaria and Cladosporium, plus 2 mycorrhizal macrofungi of the mostly poisonous fly-fungi of Amanita, and the mostly eatable Bolete fungi. Re-finds of these calibrated indicators appear to mirror the weather and climate, only when multiple bio-indicators are in a specific phenologic state. Passable ways seem to be 'co-existence indication', 'absent-indications' or 'probability indication'. A database of multi-bioproxies may contribute to a temperature reconstruction or verify the climate models.

By choosing key-organisms of the sectors, the chosen examples pinpoint aspects that may be worth further investigation:
- Compare CO_2 equivalents of the geophysics' models by reconstructing former and present temperature and precipitation e.g. from micro- and macro-fungi to achieve *another point of view*,
- Include multiple biomarkers in archeology and palaeontology from now-living indicators,
- Make operational that peak-levels of molds appear weather and climate dependent and registrations at ground level may help predict outbreak of allergy and pests,
- Awareness of crop-changes when fungi composition changes – e.g. fungi species that improve the environment for deciduous over conifers. That is based on enhanced precipitation may be at least as important for cold-adapted species as warming for these mycorrhizal tree-fungi.
- Dispersion and migration of fungi or other poikilotherm organisms may be fundament for Bioclimatic maps that virtualize changes in climate, in biodiversity and in key-organisms of the sectors. Most data of indicators and their locations are found available. They need to be systematized and when climatically calibrated, all relevant GIS-maps can be merged into animated bioclimatic maps.

Introduction

Calibrating Bio-climatic indicators – to become bio-proxies.

A methodical assessment of how to calibrate reliable indicators to workup climate-data.

Working up climate-data biologically: Before the invention of thermometers, little data was available of *in situ* temperatures, precipitation etc. Even when invented, "true" temperature of the air could not be measured realistically in free air because of diffuse radiation, precipitation or sheltering (BACC Author Team. 2008). Local precipitation data are especially doubtful remote from weather-stations, and they are poorly correlated with satellite or radar data at ground level. In these locations, we need to do our best with any reliable proxy, we may find.

The Mann's global temperature reconstruction figure was built on multi-proxies including valuable biological indicators, like pollen, forams, and tree rings, that mirror past temperature and shifts in seasons (De-Wei Li 2005; Steinman, H. 2011; Bøgh, P. 2013). Global or coarse scale climate belts' classification has been developed based on composition of plant-species and their climate tolerances from Vahl climate zones (Reumert J. 1946) to USDA Plant Hardiness Zone Map (2012), but classification based on few taxa are today regarded debatable (Ai-ping Zeng, 2011). A lot of impact models and predictions are made, about how single species may adapt, migrate or die out 50 years from now. They provide scenarios based on macro-climate change models (Gitay, H. et al. 2002). Biome models are recently used as bioclimatic indicators (Cramer, W. 2002) while a full flora (Ai-ping Zeng, 2011) and *changes* in bird *compositions* are recommended (Lindström, Å, et al. 2012). It is very rare, however, that such thorough investigations are available especially GPS-localized and temporal specified – and everywhere and whenever we need climate indications.
Local Climate Models are already in use to find the affect of extreme weather incidence (e.g. forest fire, Arif, A. 1996). Few bio-proxies, however, are available and new ones are here searched for among *living* climate-dependent species.

Regardless the objections to the 'Climate harshness hypotheses' (Gobin, J.F. & R.M. Warwick, R.M. 2006) of the 'Latitudinal gradients in species diversity' (Buckley, L.B. 2010), organisms only inhabit climate ranges they tolerate, as the "impacts of climate change are felt at the local level" (Marni Koopman & Alan Journet, 2011). This 'perceived' weather condition in *micro*scale is the 'de facto' living condition for each organism. So, local and time-specific weather-parameters may only be indicated by organisms in a short-term phenologic stage (Rasmussen, A. 2002; De-Wei Li 2005; BACC Author Team. 2008).

Statistically sufficient organisms with specific climate tolerances need to be recorded in a searchable database. That is to statistically wave aside exceptions like bio-proxies with new climate-unrelated mutations, with ethological adaptions, or species that act deviating in a special biotic interaction. *So a solution could be to indicate the local present-day precipitation or temperature from a common denominator of multi-bioproxies tolerances based on a database of living calibrated bio-indicators.*
After sufficient records in the database, re-finds of many compositions of multible bio-proxies may reconstruct a narrow range of the *in situ* precipitation and temperature for a very specific time and location.

Two aspects are considered in this rapport. Methods to calibrate bio-proxies and ways to use the bio-proxies to assess changes in climate and in communities of species. Examples of key-organisms from the sectors provide this in addition to new sector relevant information.

Usage of Bio-proxies – to indicate changes in climate and in biodiversity

That is exemplified assessments of how to 'indicate climate-changes and changes in biodiversity'. The examples are taken from key organisms of sectors.

The effort in return for calibrating the indicators are beneficial to both basic science and to the applied science sectors. Basic issues of geophysics include procedures to workup climate-parameters from selected examples to later reconstruct past and present climate data, to be patched into parallel climate models or to verify the climate models (Evansa, S.E. 2012). Basic biologic processes include how the potential indicators react according to weather or climate conditions, plus how climate affect organisms. The longest period studied is 27 years to assess possible climate-changes using data of molds microfungi and mycorrhizal macrofungi.

Cross-disciplinary, the point is to reliable study interactions between changes in climate and changes in composition of species (biodiversity). For applied science the organisms selected could derive from many poikilotherm organism – species of any tree or fish, species of tropic adapted diseases or allergic species, new crop, invasive species or pest. Although the focus is on two groups of micro- and macro fungi, their relevance on many sectors are mentioned.
As trees' uptake of water and nutrients rely on mycorrhizal fungi, any climate change of these fungi may influence the livelihood of warmer adapted deciduous trees versus colder adapted conifer trees. According to the past 27 years of changes in temperature and precipitation, indications of the organisms composition and their location are related to different adapted mycorrhizal fungi and their classified climate zone.

Beyond the historical past, paleoclimatic reconstructions have long time been used to indicate annual climate conditions (e.g. Pross, J. et al 2001) and for coarse scale resolutions using coexisting spores and pollen (Wahl, E.R. 2012). Especially bio-proxies from the marine environment, such as corals foraminifers or diatoms, have been utilized (Henderson, G.M. 2002) to indicate salinity, temperatures and even sea levels (Horton, B.P. 2007). A few terrestrial investigations exist to indicate past UV radiation (Rozema, J. et al 2009) and population sizes (Gill, J.L. 2013). Few Bio-proxies are available, even though most temperature proxies appear reliable (linear), Støve, B. et al (2012). Sometimes, it is doubtful what indicators, in fact, indicate. This study screen for supplementary methods to provide and combine now-living indicators to narrow down a local and time restricted temperature and precipitation range in the land areas – the first areas affected by climate changes. The supplementary methods are co-existence indicators, absent indicators and probability indicators, plus comparison between local communities and those classified according to their climate zones. The chosen now-living indicators are well-known to archive as spores, and when calibrated, they may be suitable for paleoclimatic reconstructions, too. Whenever reliable indicators of daily precipitation are unavailable, we strive to define what is, in fact, the perceived water tolerance of alternative indicators of the flora (a waterlogging value).

Further to this study, other accumulative residuals of calibrated indicators may mirror past climate conditions. Any time dating is applicable (Anchukaitis, K.J. & Tierney, J.E. 2013), climate changes of organism may be compiled into fine gridded maps location after location. These climate indicated localities may together with soil resource maps, topographic maps etc. be patched into fine-gridded and coarse-gridded bioclimatic maps. When long-lasting biomarkers are achievable (like the hard-shelled spores), likewise detailed maps back to paleontologic time may be developed, as well. Animated bioclimatic mapping through time and space may likely be developed.

Methods

Choice of geo-physical data in the study:
Daily mean temperature and daily mean precipitation at ground level are the best available data due to inconsistent recording throughout the studied period.
When a mixture of weather parameters appear decisive a restricted parameter are defined, the waterlogging value. After precipitation, the soil is fully saturated (high relative waterlogging values) and although immediate drainage and evapo-transpiration occur some water is held back for days especially as the soils of the area studied are all clayey moraine soils that gradually dry out until soil hysteresis.
The influence of the remaining water on the organisms is estimated from near-past precipitation and time of drought: A 'relative waterlogging value' is appointed from the accumulated water-content in the soil after each day's precipitation (daily mean registered). That is we define

1. The first day of each year with ≥10mm of precipitation, the soil is presumed soaked and the waterlogging value is set to 10. After any new precipitation ≥10mm the value 10 is given again.
2. When precipitation > 0mm, but at a lower value (<10mm), the dry out is presumed stopped for the day. And any dry out the next day is calculated from the level of the day before:
3. At precipitation<10mm the value [1-precipitation/10] is subtracted. (E.g. 1mm dry out per day if no precipitation) until presumed dry out - and the value reaches 0.
4. The relative waterlogging value is relative to the highest value registered (during the 27 years of the study). It is set to 100% and the lowest value (of the 27 years of the study) is appointed 0%.

Most data-series were designed to investigate other aspects such as Road weathering modeling or allergenic assessment leaving out other climate data. Moreover, weather-stations have been moved, the scaling of reconstructed data has changed, radar and satellite data has replaced manual monitoring. Especially finding local precipitation data may be inadequate. So a consistent correlation of the potential proxies to the physical data is implemented in the immediate vicinity to a permanent weather-station.

Choice of potential bio-indicator species:
→ Statistic acceptable data-series:
To find data and make compromises between enough bio-data in a small area to be statistical reliable and homogeneous weather-data are demanding tasks. Obvious reliable number of species (the micro-fungi) or merged species (climate zone lumped species) are chosen to gain enough replicates with short development cycle and similar tolerances. Besides, the choise of microfungi in their extreme dispersion level secure that distant specimen from deviating climate conditions are redundant compared with the major number of local developed fungi. The location of the macrofungi are in the Copenhagen-North Sealand region with the most frequent gathering of fungi and so are the statistic foundation.
A precise geo-location and sampling time are needed to correlate local climate data with the biological tolerances. *Calibration* of the bio-indicators is carried out by corelating species appearance in a 5.3km^2 area with momentarily weather data. The calibration is based on lumping the last 9 years to gain an acceptable foundation. It presumes that the climate has not changed and so the number of organisms in this short period.

Different organisms may more or less reliably indicate different parameters and climate ranges. Normally, there need to be multiple indicators to delimit a narrow tolerance-interval. Two very different and reliable indicators may together narrowing a range ('coexistance indication'). If the indicator is calibrated in a stage when it is numerous, a missing indicator at this level may exclude a tolerance range ('absence indication'). Finally the most well-calibrated indicators, due to a good statistically foundation in various areas (like microorganisms or spores), may indicate their tolerance range from the probability of being present in their locality e.g. 95% of the time recorded ('probability indication').
Long term data series are useful to indicate climate changes. However, very few biological datasets

are sufficient or have continuously been gathered throughout a climatological time scale of at least 20 years. Especially few has been geo-located and date-marked, which makes the datasets difficult correlatable with their climate tolerances. After calibration, 27 years of Alternaria and Cladosporium are studied to find if any 'long-term' climate-change may be visible. The final challenge is to find the criteria of when the indicator organisms in fact mirror their surrounding climate:

→ Change in ethological or genetical adaptation:
However, if organisms change behavioral response to the climate or change genetics during the time of the investigation, the indication may bring mislead reconstructed climate data. To avoid errors due to behavioral changes, literature of resent 'ethology' studies can be included. When multi-proxies with the same weather tolerances is included in the study, it is possible to disregard genetic adaptation, as several mutations the same time are highly improbable. Finally, human influence that periodically change composition of local indicators or their phenology of a number of the indicators may disrupt the reconstruction of the local climate data. In practice, however, carefully choosing the habitate and adding a high number of organisms to the correlation procedure may overrule misleading outliers. As a bonus, any new adaptation of a species found, will add interesting biological aspects to be studied.

→ Climate affinity of the chosen species
To choose a climate indicator, any distinct climate affinity of the organism is valuable.
It is time-consuming to look for a great number of potential decisive climate parameters. For this screening of possible indicators, however, we rely on possible indicators mentioned in the literature that provides multiple regression of decisive parameters, in which temperature (and precipitation) often comes in first (Selenkovi, I. 2009) especially for the fungi (Sousa, S.I.V. et all 2010) - as the studied area has no elevation. The chosen microfungi (Altenaria and Cladosporium) are cosmopolitan species but their spore dispersion deviate from between climate zones (DK has one autumn peak period while Turkey has two (Celenk, S. et al. 2007). In this study, the Amanita fungi are characterized as Cold-adapted, Semi-warm adapted, Warm-adapted and Indifferent species. This is based on where they are common in Boreal, Hemi-boreal and Temperate areas or are climate-zone-indifferent species; likewise, Bolete fungi are classified and lumped according to their climate-zone affinity[Table 1,2].

→ Phenologic stage of the organisms
Bio-indicators that mirror the *in situ* weather throughout the period investigated are preferable. Organisms may immediately respond to precipitation by finding shelter for rain like insects and birds to find a beneficial microclimate. Most species respond to their perceived air-temperature like the poikilotherms. In special periods, as in the breeding season, organisms need to expose themselves to the current weather: like fruit bodies of fast growing fungi and microfungi, when they reach a suitable threshold of moisture and temperature to spread their spores. Besides for weather-indication, an increased level and change in composition may directly indicate changes in climate. Alternaria and Cladosporium spp. fungi are chosen, as microorganisms appear in large numbers. In addition to good statistics, the same method is used as for pollen, Burkard 7-days recording volumetric spore traps at DMI throughout 27 years.

The corresponding geolocation is precisely defined, which helps to correlate with the local weather-station. When mature, these microfungi produce their spores immediately (in contrast to pollen). According to Ai-ping Zeng, (2011), dispersion of Alternaria spores are expected to occur during dry periods. These feature higher wind velocity and lower relative humidity, which result in peak-level dispersion during sunny afternoon periods (Steinman, H. 2011). All that is expected to mirror the climate if any correlation exists. However, despite the same large spore size of Alternaria as pollen, individual Alternaria spores may "disperse for hundreds of miles from the source. Counts of Alternaria on dry, windy days can be in the range of 500 to 1,000 spores per cubic metre in grass-

or grain-growing areas” (Steinman, H. 2011).
To face these point, the focus of these cosmopolite and pioneer microfungi is on their sudden peak-level dispersions. The *peak-level* choise of the fungi are chosen to ensure that most of the spores are unlikely re-suspended from long distances, but are dispersed locally. Besides, choosing the same peak-level that affect allergy may enlighten important allergy issues:
An "air concentration of Alternaria spores exceeded the limit value of 100 spores per m3 ... is associated with increased risk of allergy" (Prester, L. 2011). A likewise reasoning is behind the choice of the Cladosporium: "A 10,000-spore/m3 increment in Cladosporium spore concentration was associated with a deficit in morning PEFR" (peak expiratory flow rate, Neas, L.M. 1996). They are registered at DMIs weather-station in Copenhagen (55.7154 N x 12.5609 E) and the peak-level of spores derive from a radius of 2-3 km from DMI, where the agriculture has not changed throughout the 27 years of the registration. And more than 95% of the spores belong to the two species *Cladosporium Herbarium* and *Alternaria alternata (*Suldrup Larsen, L. 2011).

Finally, a Clostridium (*C. botulinum*) is found by Krzysztof, F. & Dariusz, R. (2011) to last for several years in soil and some Cladosporium spp. spores seem to be found even as fossils (Krumbein, W.E. 2003). Such achieving leaves reconstruction possible. However, *Alternaria alternata* "(leaf spot) produces spores, which are often poorly preserved” (McAndrews,j.h. & Turton C.L. 2009)!?
Choosing the peak-levels are to find 1. any regularity to spore dispersion and climate parameters. And even though each years dispersion peaks at different times[Fig.1], we'll like to find 2. if the spore dispersion still happens within a specifiable season that may change according to climate changes, and finally 3. assuming the prognosis of increasing incidents of summer warm, moisture and extreme weather is valid - how have these incidents affected the spore dispersion and so the possible agriculture pests / allergen outbreak the last 27 years?
Different phenologic stages such as microbial sporification of molds and macrobial fruiting of mycorrhizal fungi have been chosen for the indicators.

Sporification details of year variation of Alternaria and Cladosporium (Archieve at [DMI.dk](DMI.dk)).

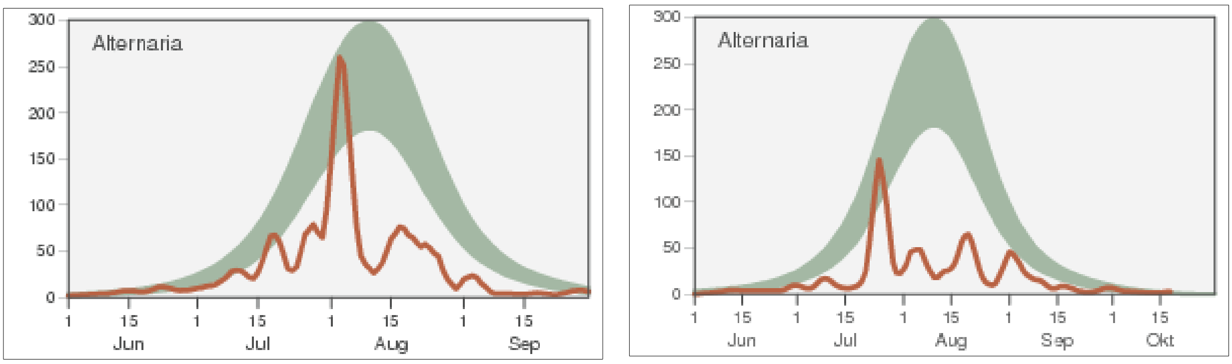


Fig.1. Even though 90-99% of the spores of Alternaria and Cladosporium derive from single species, Cladosporium herbarium and Alternaria alternata, sporification registrations does variate from year to year (e.g. 2011 and 2012). However, during the 9 years of the calibration study, no significant pattern of climatic changes is found. For that reason we merged these years into one dataset.

Fungimap Bulletin (May, T. 2010) provides a survey of how macrofungi depends on the weather: "Rainfall is vital for fruit-body production. However, temperature also seems to have a role, in two ways. Firstly, in relation to the soil needing to be warm enough to allow hyphal growth and secondly because high temperatures cause evaporation of moisture from soil, reducing the available moisture. Soil temperature might only be important in colder regions (such as alpine areas) but high evaporation could be a limiting factor in many micro-climates they inhabit. The interplay between rainfall and temperature in determining fruit-body production will likely vary for different species, as evidenced by presence of fruit-bodies of some genera such as Amanita after rain in spring and summer. Phenology patterns will also vary for different climatic regions, and across soils with different water-holding characteristics. There are also other variables that might affect fruit-body production, such as the metabolic state of the plant partners of mycorrhizas fungi. "Both for Amanita and the Bolete fungi, a short fruiting cycle of about 3 days with a mean daily temperature and precipitation is correlated with the number of fungi reflecting the decay: The Amanita muscaria var. alba sporification decline after three days "(the concentration of spores dropped by 95 %)" and the "Boletes discharged their basidiospore continuously for 1–6 days" (De-Wei Lia & Kendricka, B. 1995). The macrofungi chosen belongs to the genus *Amanita* and the '*Bolete*' fungi belongs to the genus *Boletus*, *Leccinum*, *Tylopilus*, and *Suillus*. The macrofungi represent species with a distribution optimum of several climate zones[Table 1,2]. That way, they are expected to tolerate and reflect different local weather conditions. They are registered in Hareskov-Horserød, a 'regional' area of 57.5 km² (55.4-56.1 N x 12.1-12.5 E). To compare with this regional area, a 'local' scale area from 5.3 km² is chosen with the same center in Copenhagen as the one for the microfungi.

→ Choosing the indicators among key organisms of sectors:
The choices of organisms for indication in this study may expose climate-change information that is important for key organisms of the sectors. In this study:

Climatology: the point is to screen the options of how to find reliable bio-indicators to eventually get another point of view to verify the climate scenaries or supplements of former and present climate parameters (e.g. local temperatures to reconstruct CO_2-equivalents for the models).

Palaeontology & Archeology: Spores of fungi are evident in protected sediment, rosins, ice and fossils. Especially the chosen Cladosporium fungi in peak-levels may tolerate certain temperatures and so elucidate historic anthropological changes in land-use or even palaeoclimatic processes. Provided that the well-calibrated fungi consequently do indicate their climate tolerance, their surrounding precipitation and temperature can be clarified. Alternaria is attached to open land grasses as cereals and the 'brown leaf spot pest' of potatoes, while the other chosen Amanita and Bolete mycorrhizal fungi are associated to trees. A shift from the open land species to tree associated species may indicate a shift from Stone Age forestry to agriculture - or indicate the time of reheating after the little ice-age.

Agriculture & Forestry: Any dispersion of Alternaria related to the climate will be relevant for pest management and choice of crops. Likewise, any climate changes in species composition of the studied mycorrhizal Amanita and Bolete fungi may affect the tree production and choices of deciduous trees over conifers.

Landscape management & Conservation needs: The microfungi and the mycorrhizal fungi may indicate very time-specific parameters of the micro-climate. This may indicate that the area is cold, while others are warmer. In the different bioclimatic groups (of the studied Amanita and Bolete fungi) some species may appear indifferent to temperature, while others tolerate a fixed temperature range. Knowing how common the present adapted fungi are and information on the former and future climate may give a hint of who may be decimated or who may benefit from climate changes.

Healthcare of allergy: Allergy warning of the main airborne allergens, the chosen Alternaria (A.) and Cladosporium (C.), are developed to prevent allergist to be exposed to peak-levels of these fungi spores. The allergenic peak-levels are chosen exactly at the level of allergist deficit in the morning PEFR to discover regularity of allergy outbreaks related to weather and climate. The dispersion process of other allergenic agents, like pollen, may be related to A. and C.

Public climate- & nature-awareness: The present study may affect the awareness about changes of climate and nature due to the major impact on personal matters. Such changes might be in the allergic level (of Alternaria and Cladosporium) or just revealing biodiversity experiences (of poisonous fungi, like most Amanitas, or eatable Bolete fungi).

Results

Bio-proxies to workup local and time-specific climate data

The criteria of a well-calibrated bio-proxy

When organism's phenology, such as reproduction, suddenly change in relation to 'season', it may actually respond to a changed 'micro-climate'. Changes in spore dispersion are found for Alternaria and Cladosporium(Fig.2). The challenge is to access which parameter(s) that is decisive for the right 'perceived' climate for each species.

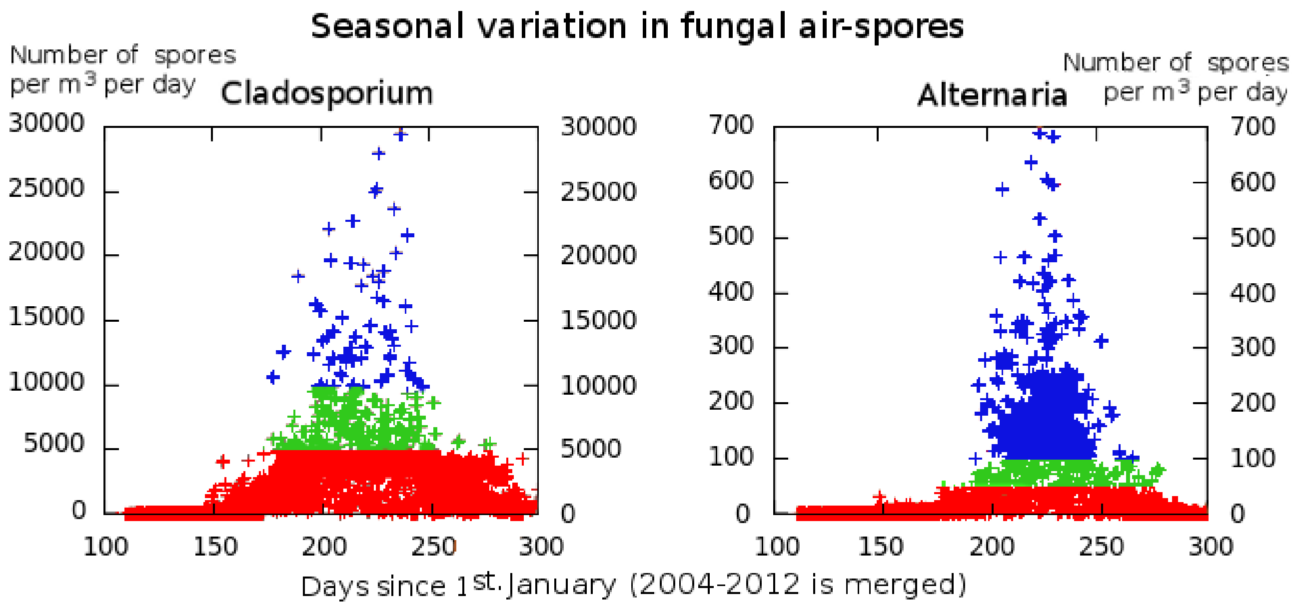


Fig.2. For Caldosporium and Alternaria, 10.000 and 100 spores /m3 per day, respectively are given as a maximum critical level for allergists (morning PEFR). When merged through the 9 years of calibration, the actually found peak-level seem to coincide with a definitive 'season'. A special season often means a specific weather constellation (temperature, precipitation etc.). The (allergenic) peak level of spores are here studied according to their climate to correlate with local dispersions.

The calibration of these indicators need to take into account that they actually reflect their surrounding climate directly or indirectly. When organisms are significant more frequent in one season than another, one or more climate parameters may be decisive. If one parameter is absolute decisive, the indication is valid. The Semi-warm adapted Amanita fungi(Fig.3) are found at any precipitation and only at high *temperatures*. So below, this group of fungi is studies further below. If an indefinite combination of climate parameters is decisive, the species are useless indicator.

Changes in composition & distribution of species and their season

Timing, however, may be relevant: Amanita species grouped as 'Indifferent spcies' according to their climate-zones seem to develop fruit bodies in any combination of temperature, precipitation and season (Fig.4,5). When the season is right, the indifferent one indicates unknown decisive parameters. It make them appear as generalists and make them useless to indicate climate parameters. It is in contrast to Semi-warm adapted and Warm-adapted Amanita that appear to reflect 'seasonal changes' that again may be due to the right temperature. Even if the variation reflects a succession of the organism, the succession itself may be a consequence of a climate adaptation and indirectly mirror a climate parameter.

Without a multiple correlation analysis, we rely on the literature to focus on temperatures and precipitations(Fig.6). It appear likely that temperature and not precipitation is decisive for the Semi-warm Amanita fungi. It is supported by looking at the other fungi related to temperature(Fig.7). The Warm adapted Amanita fungi do not equally appear in early summer and late summer even though the temperature is the same. It indicates another parameter(s) to be decisive.

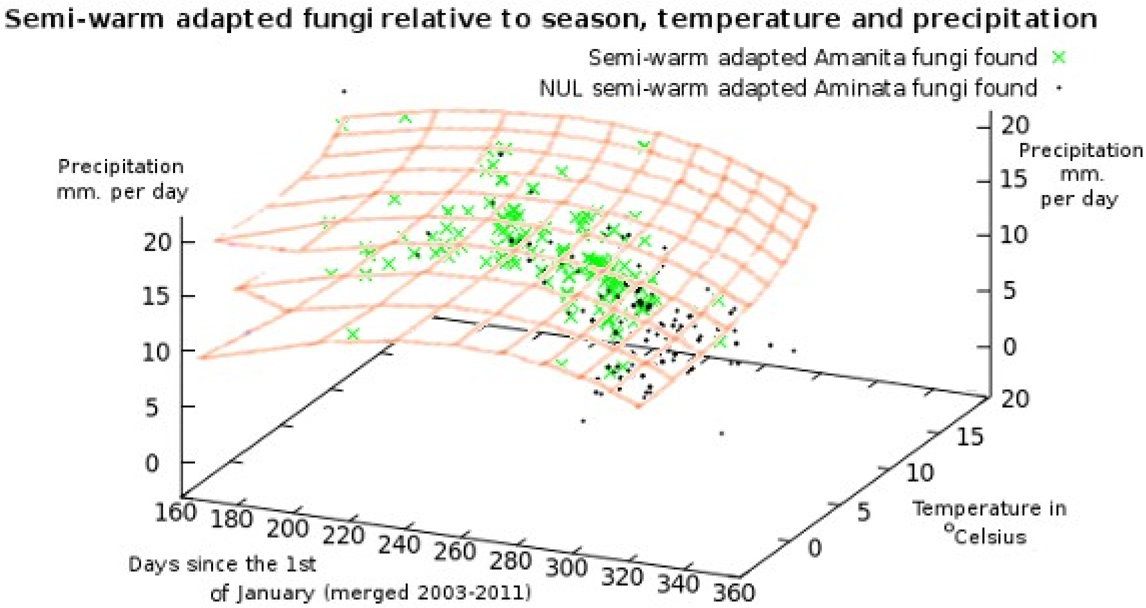


Fig.3. The Semi-warm adapted Amanita fungi are found until Autumn at high temperatures (red network) and at any precipitation studied. Apparent seasonal variation gives hope for a parameter to be climate related.

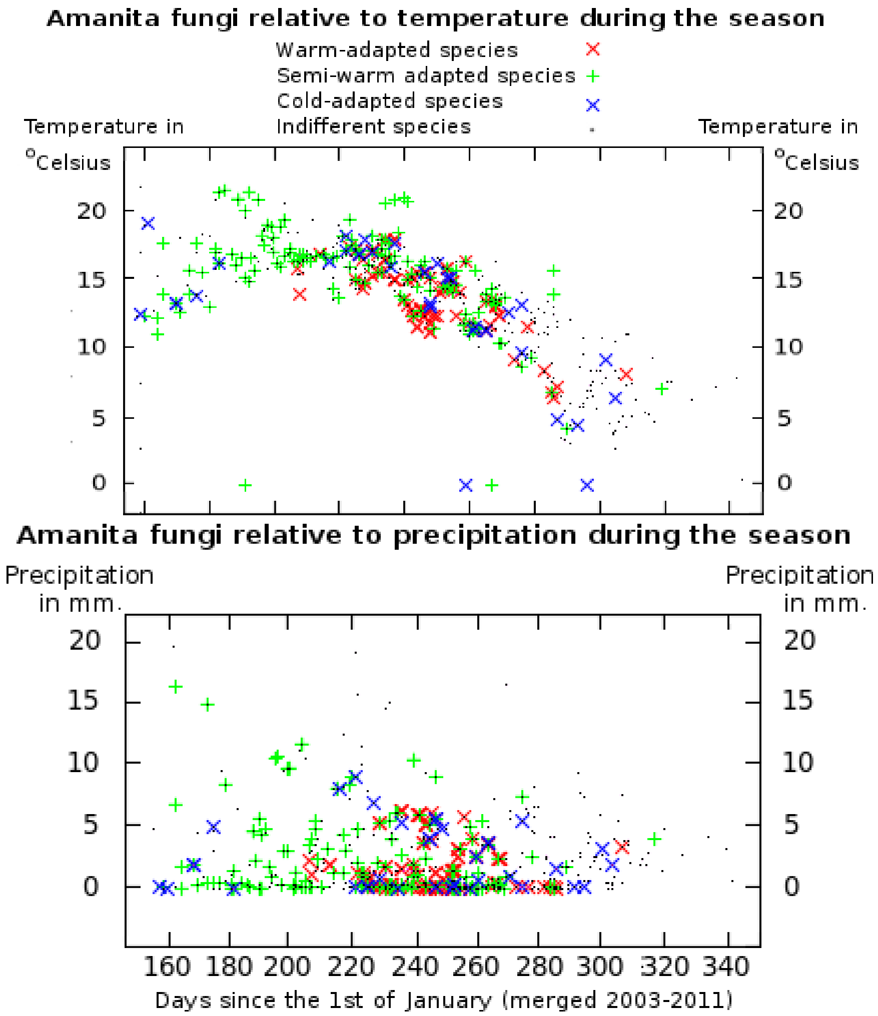


Fig.4. and Fig.5. Changes in presence may indicate climate changes. Many species defined in this study(Table 1) were classified as 'climate-zone indifferent species'. Only 2 incidents out of 1533 dates, no registrations of the 'clima-zone-indifferent' species were found. So, in this location, they do appear indifferent regarding temperature and precipitation. The Warm-adapted Amanita species seem to start at 210 days while the Semi-warm adapted Amanita appear to stop fruiting at 290 days.

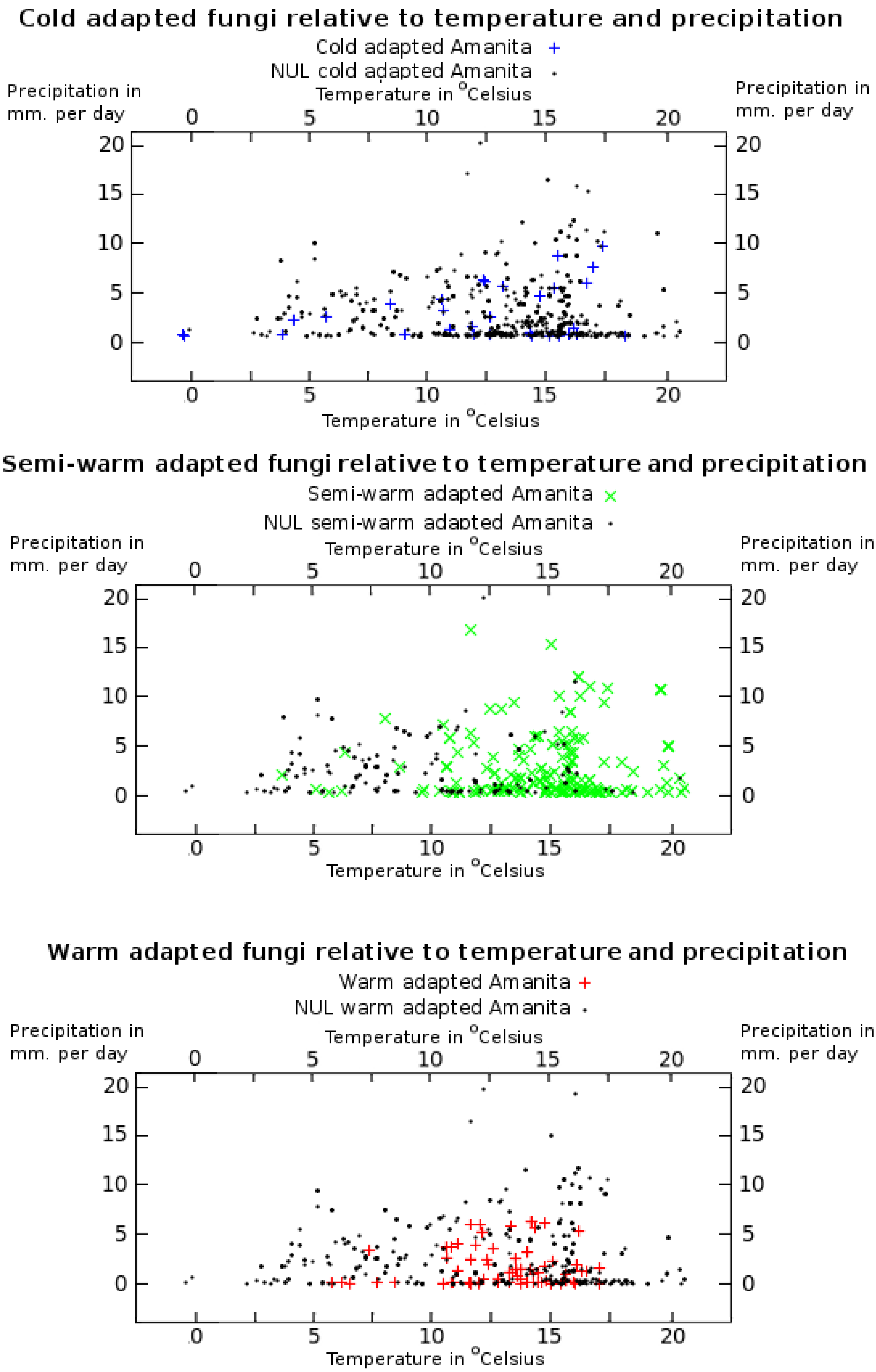


Fig.6. The semi-warm adapted Amanita seem to be cosmopolitan in the respect of precipitation, while the temperature seem be decisive above 10°C. The others seem restricted to a precipitation below 7 mm per day and as all Amanita fungi, they seem rare below 10°C.

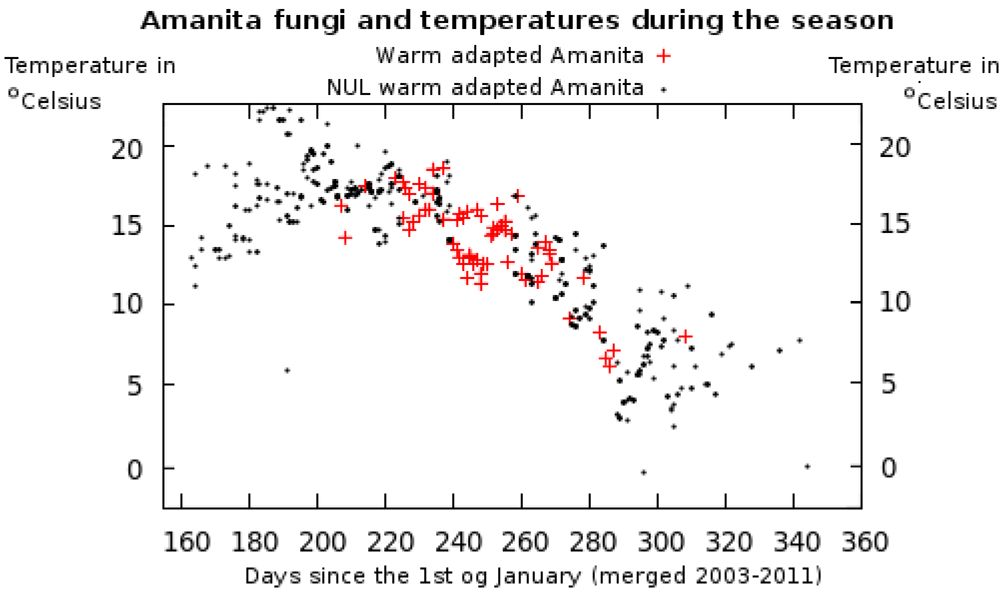


Fig.7. A useless temperature indicator: The group of the 'warm adapted' Amanita fungi are most common in the temperate climate zone studied(Table 1). IF temperature was decisive, the same fungi growth would appear in spring as in late summer at e.g. 14˚C. A combination of climate parameter could be decisive (such as waterlogging described below).

Indicating a narrow temperature range

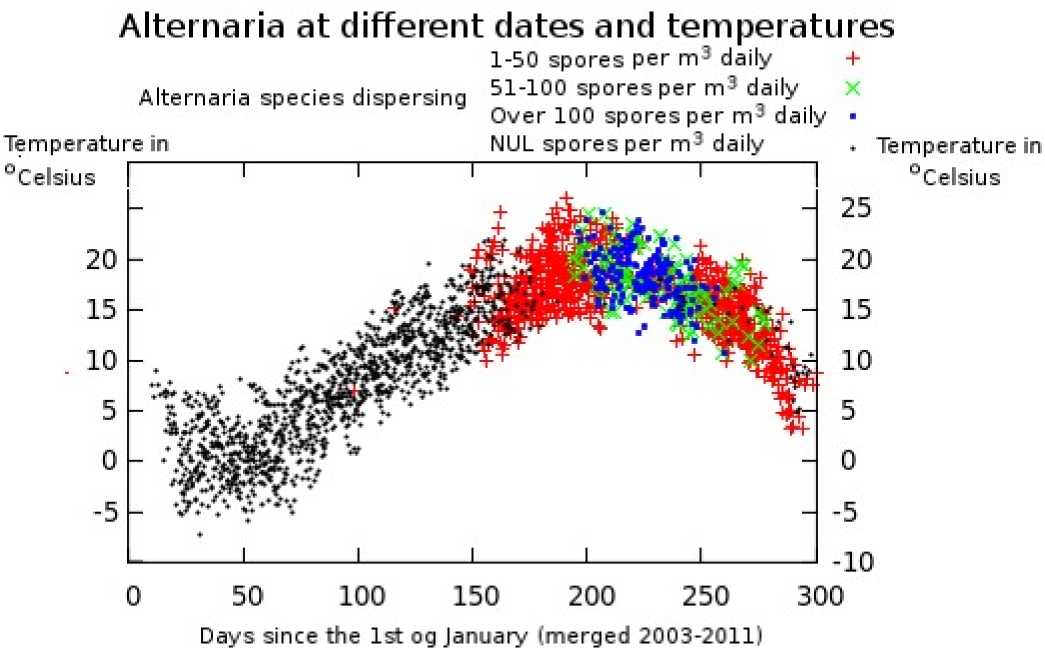


Fig.8. Spore levels from different time of the year seem to disperse according to periods which may imply that they have certain temperature tolerances to disperse at 15m's altitude.

The peak-level seen in Fig.8 over 100 spores of Alternaria are extracted and are utilized for further study. Peak-level dispersion of Alternaria is found in autumn when the temperature is above 11°C(Fig.9) and Cladosporium has the same pattern(appendix Fig.23). The distribution of the peak-level seem delimited to the same temperature range, when repeating and correlating the number of spores with temperature for all the Alternaria registered throughout the 27 years.
In the study, no spores at peak-level (above 100 spores $m^{-3}day^{-1}$) is found below 11.2°C. Despite it looks like a normal distribution (when peak-level is regarded). No statistic analysis is carried out because the daily average temperatures above 24°C are very rarely registered in Denmark. So it's not possible to claim a decrease of the spore dispersion at high temperatures due to the *tolerance* of the microfungi.

In this screening, 2 dominant species of the air-borne microfungi and 37 fruiting macrofungi are chosen. Out of these, 6 seem to be possible indicators. These are compared with the non-decisive species of related fungi(Appendix Fig.26,27).

Fig.9. A peak-level dispersion of Alternaria spores may indicate a temperature range

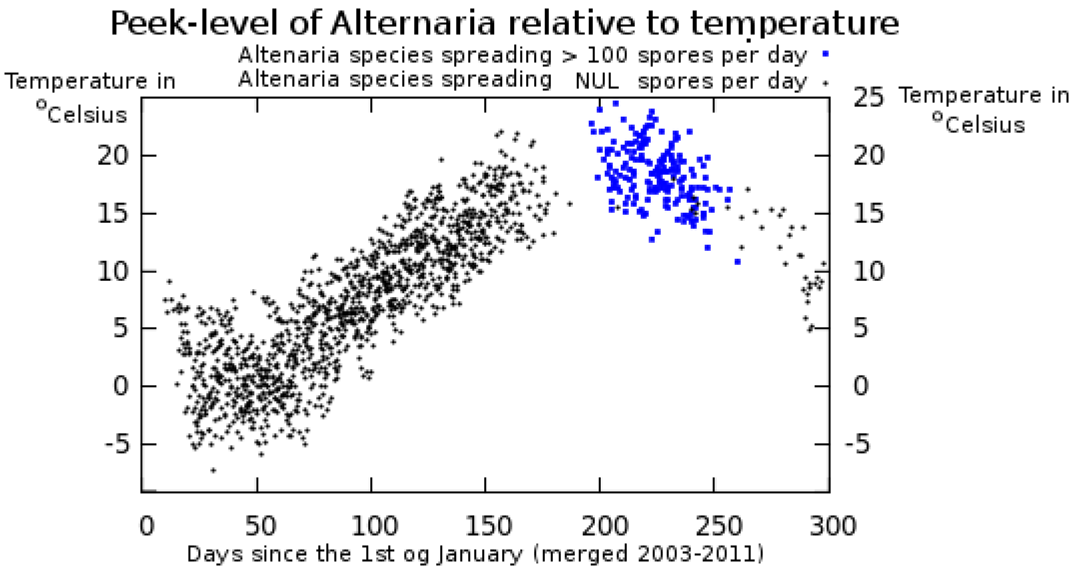


Fig.9. The peak-level of Alternaria (>100 spores per m3 per day) are cut out and they seem to indicate temperatures above 11°C and Cladosporium (>10000 spores) above 13°C(Appendix Fig.23).

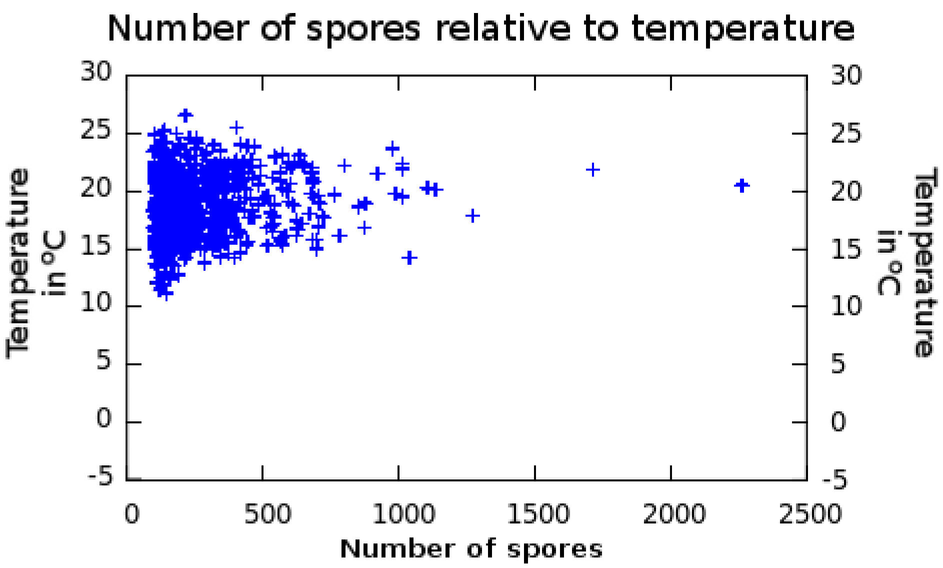
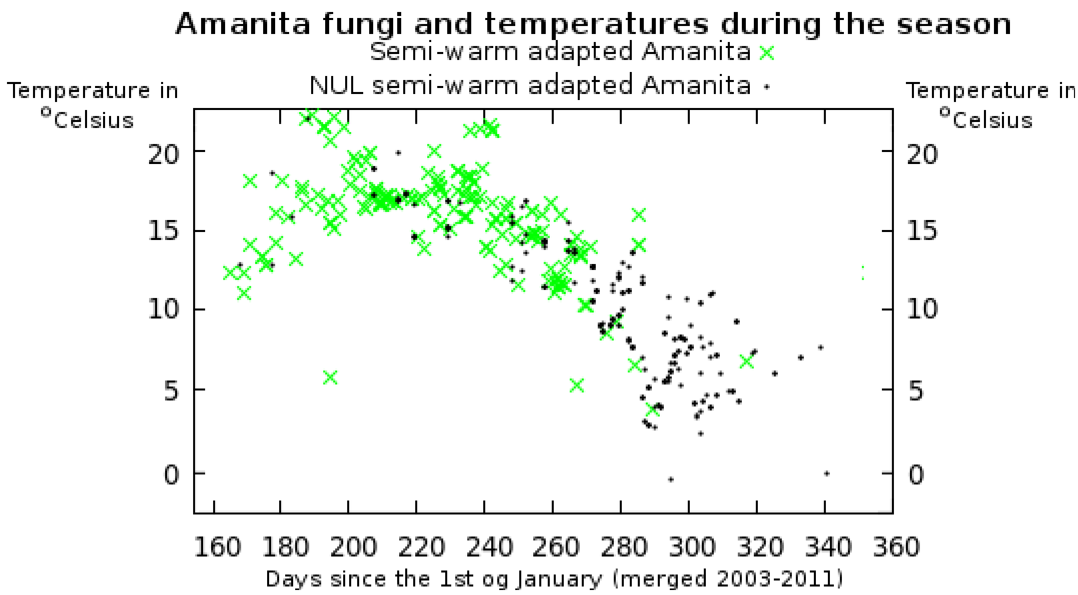


Fig.10. After calibration (2003-2011), all peak-level data of Alternaria(>100 spores per m3 per day) available for 27 years are entered into this temperature/number figure. This confirms the temperature tolerance range.
No spores at peak-level (above 100 spores $m^{-3}day^{-1}$) is found below 11.2°C in the 27 years of the study; that is independent of season. If your allergy tolerance is 100 spores per m3 per day, you'll know the minimum temperature, too. The most probable outbreak of allergy (>95%) are found above 15°C.



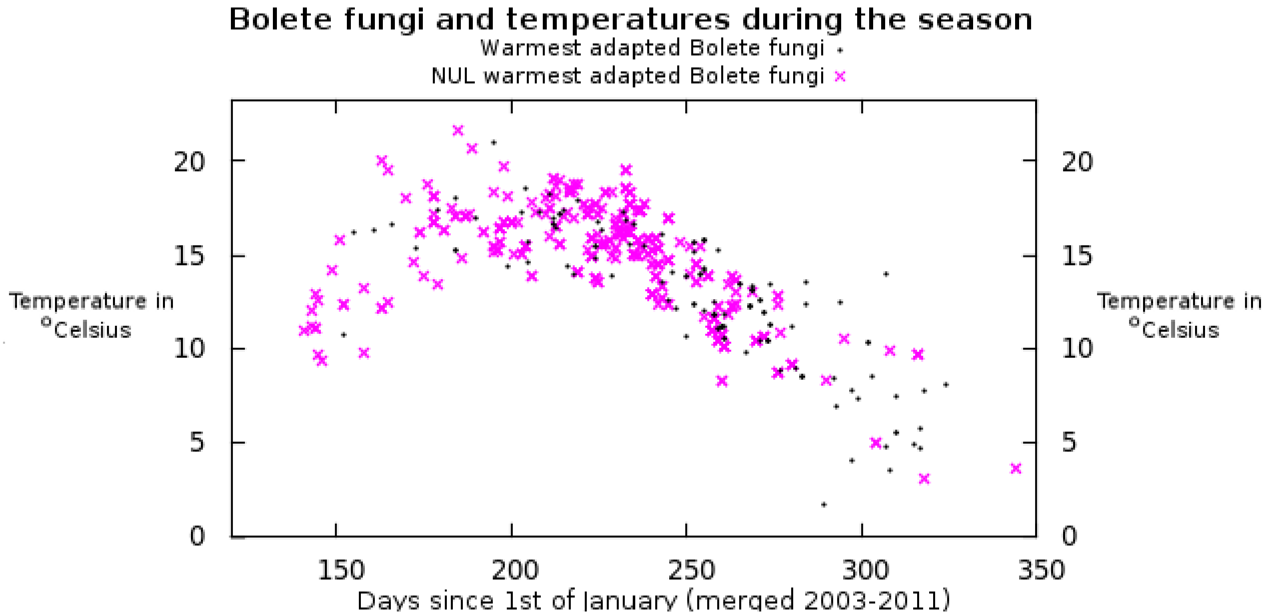


Fig.11. The Semi-warm adapted Amanita and the Warmest Bolete macro-fungi appear to indicate their habitat temperature above 10°C. They seem dependent of 'season', they appear equal distributed at the same temperature (spring and autumn) and at least the warmest Bolete fungi are found more frequent above 15°C than below(Table A)

Out of all the recorded fruiting fungi, the group of Semi-warm adapted Amanita and the Warmest Bolete fungi are found to be most possible temperature indicators(Fig.11). The NUL registrations are remarkable not-absent at certain temperatures.
If we sort the Bolete fungi by temperature in two equal fractions of ca.150 records and notice the climate zone they are classified(Table 1), the following numbers appear:

Table A.

Temperature	*Cold & indifferent*	*Semi-warm*	*Warm*	*Warmest*
0-l4.9°C	98	175	152	164
15-30°C	70	137	142	227

This support temperature to be decisive for the warmest-adapted Bolete fungi compared with the other adaptations. It is visualized in Appendix(Fig.26).

The registrants of the fungi are especially aware of the (often) poisonous Amanita and especially the eatable Bolete fungi, when they record the many different fungi into the Danish Fungus Atlas registration (Heilmann-Clausen, J. et al. 2013). The 'Climate harshness hypotheses' (Gobin, J.F. & R.M. Warwick, R.M. 2006) highlight that species may be absent despite the climate zone is right for an organism. That means, being absent does not imply that the climate condition could not be suitable. Therefore, absence of *fruiting bodies of macro-fungi* may not be safe to use for indications. Spores of the microfungi, however, derive from multiple mycelia of cosmopolitan (decomposing) microfungi, and it is "a matter of frequency rather than presence or absents (Hyde, K. 2001). Registration of absence of the peak-level of Cladosporium and Alternaria are taken as a fact. These data are used in Fig.12 together with presence-registrations (e.g. from the Fig.10 and Fig.11). That is we allow presence registrations (*multiple co-existence indications)* for all fungi and include 'absents' registrations (a*bsents indications*) for statistical well-founded microfungi, spores, pollen etc. To visualize the delimited temperatures, the fungi are added to[Fig.12] below. A '*co-existence indication'* is used when it is possible to narrow down an intersection of temperature tolerance in an area between two or more species or group of species. A *probability indication* based on Statistical occurrence analysis will expose the frequency of a species for different temperatures. To be interesting and liable for modelling, however, there need to be multiple indicators, and their spatio-temporal distribution need to be analyzed statistically.

Distant genetic related organisms (taxa) with different temperature affinities may coexist in an area, and together indicate the temperature range. In the example, the indicated temperature range would be further narrowed down in a reliable way, when the Cladosporium and Amanita or Bolete fungi are registered[(Fig.12)] in exactly the same procedure, location and time span.

Extreme weather conditions may need to be indicated by single species.

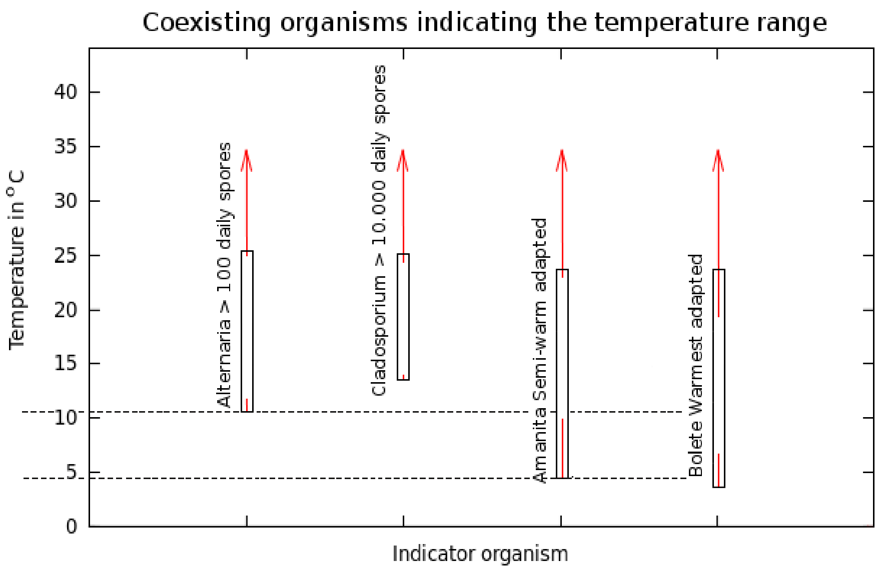


Fig.12.
'Absents indication': If Alternaria (above 100 daily spores per m^3) is not found, while the Semi-warm adapted Amanita is found, then the habitat would be between 4-11°C.
'Probability indication': In the example, when a common denominator of the Alternaria (above 100 daily spores per m^3) and the Semi-warm Amanita are most abundant (regardless a few specimens), the habitat is probably 9-11°C. Red lines in the box present temperatures that we cannot exclude due to a few specimens are found. Red arrows show that there is not enough data to eliminate that the fungi could appear at these higher temperatures.
'Co-existence indication': This indication is especially relevant when a registration of unrelated taxa, e.g. Clostridium at peak-level in an area, and the Semi-warm adapted Amanita are present together. It could indicate that the temperature on the given date may be between 13-23°C. Two indicators, alone, rarely provide a reliable narrow range; especially as it is uncertain whether the fungi can be found above 23°C.

Indicating a narrow precipitation range

Any decisive parameter beside temperature? The temperature of 9-20°C is found both in early summer and in the autumn. The Semi-warm Amanita and the warmest-adapted Bolete fungi are found in both periods. Except for these fungi, other decisive parameter(s) than temperature may be essential. In early summer, 24 days have 0 precipitation in the 3 days prior to the registration out of 96 days (25%). It is remarkable in the light of 58 days out of 440 days (13%) have no precipitation in autumn. The average precipitation, when it came, is also lower in the summer (3.60mm) than in the autumn (4.52mm). Adaptation to long term drought and lack of dispersion due to decaying precipitation may be the reason for the low number of the other macrofungi.

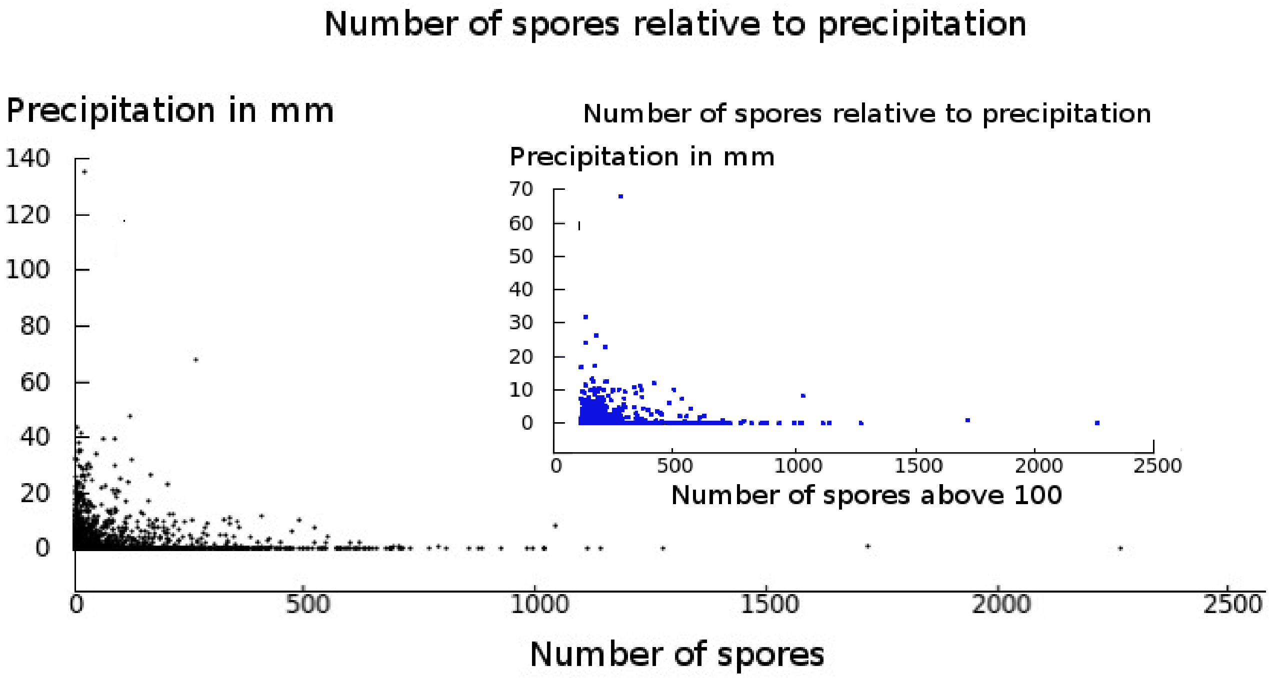


Fig.13. The less present-day precipitation, the higher spore-dispersion. A peak-level number of Alternaria spores (above 100 spores/m^3 per dag in 15m.s altitude) may appear, when the precipitation is below 13mm per day.

High level of Alternaria spores mirrors a low present-day precipitation. That is when elevated in15m above ground level (Fig.13).

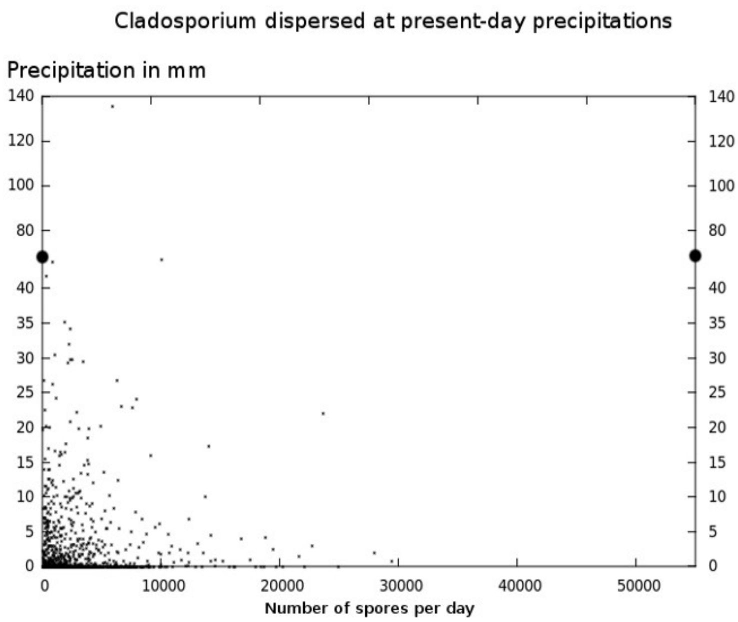
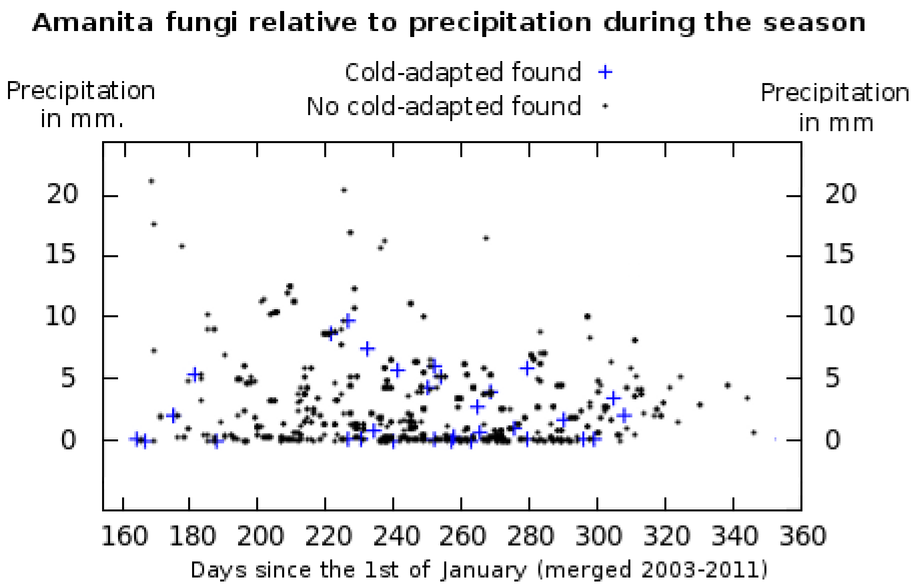


Fig.14. No peak-level dispersion (above 10.000 spores $day^{-1}m^{-3}$) of Cladosporium is found, when the precipitation exceeds 23mm.



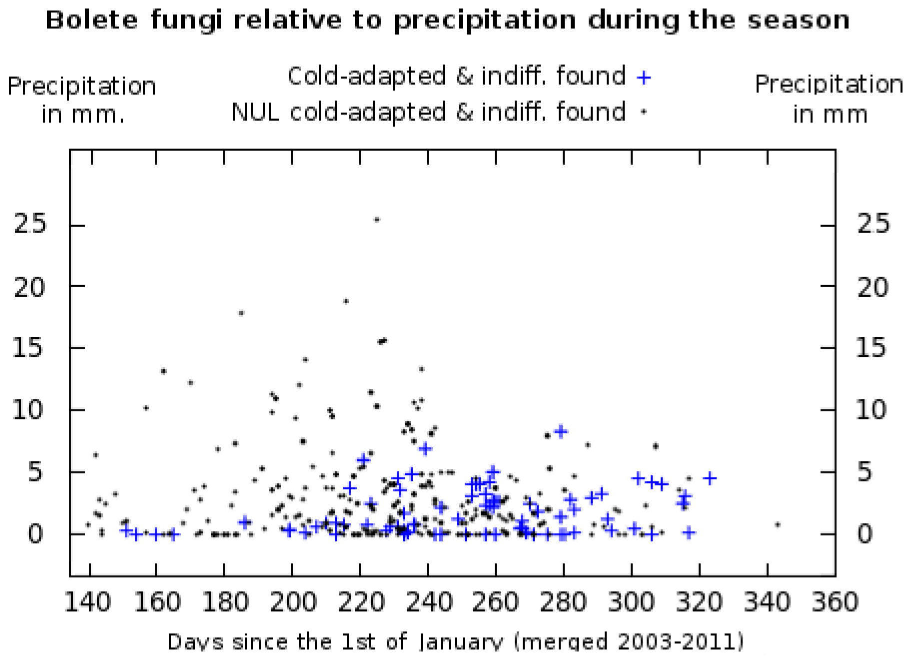


Fig.15. While the temperature does not seem decisive(Fig.7), the cold-adapted Amanita (at 0-9mm precipitation) and cold-adapted Bolete fungi (at 0-8mm) are only found in a limited range of precipitation.

The cold-adapted Bolete fungi are still common in subarctic and they appear indifferent to temperature(Appendix Fig.26) and climate zones(Table. 2), but they appear restricted to a daily precipitation below 8mm. throughout their 3 days of their fruiting development.

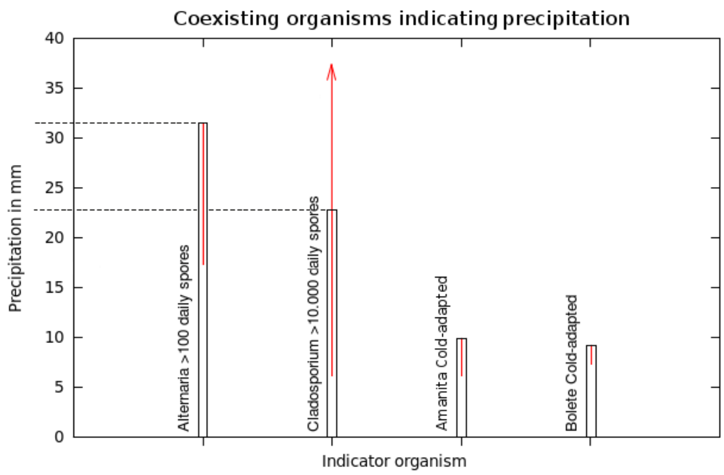


Fig.16. Both massive sporification in 15ms' altitude and development of fruit bodies are found at low or even no daily precipitation. If a peak-level of Alternaria spores (above 100 daily per m3) disperse AND less than 10.000 Cladosporium spores are found together, it seems probable that the precipitation belongs to the rare situation of 23-33mm in this locality.

Indicating a combination of climate parameters

Indicating a narrow waterlogging range

Parameters beside temperature and precipitation may become decisive as peak-level dispersion at high precipitation and low temperature does occur - especially for plants and fungi. Waterlogging is 'the history of the thermal precipitation' or remaining water – probably at ground-level.

A correlation is found:

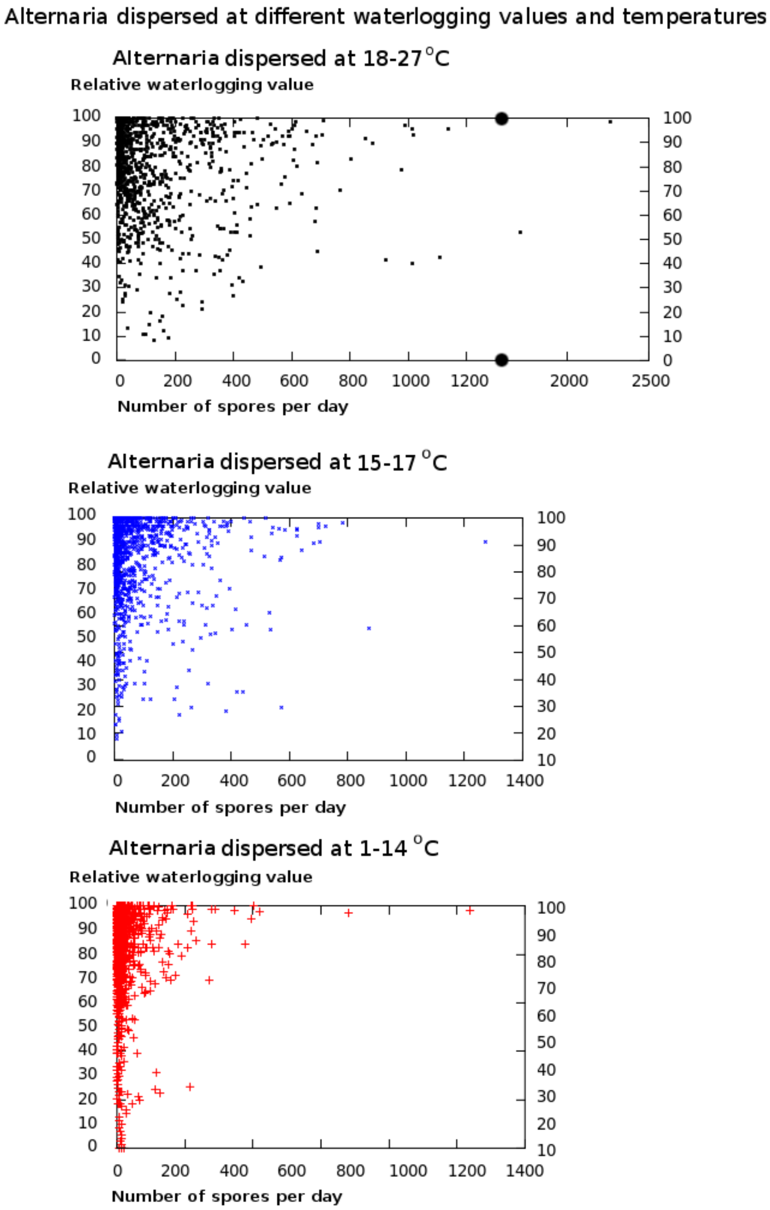


Fig.17. In contrast to the high dispersion at low precipitation, the Alternaria seem to disperse evenly, or when the remaining water – the waterlogging value (WV) is high. Especially at high temperatures and high WV, a scattered dispersion still proceeds, which may be due to a high spore-production at ground level. This also apply to Fig.18.:

Clostridium dispersed at different Relative waterlogging value and temperatures

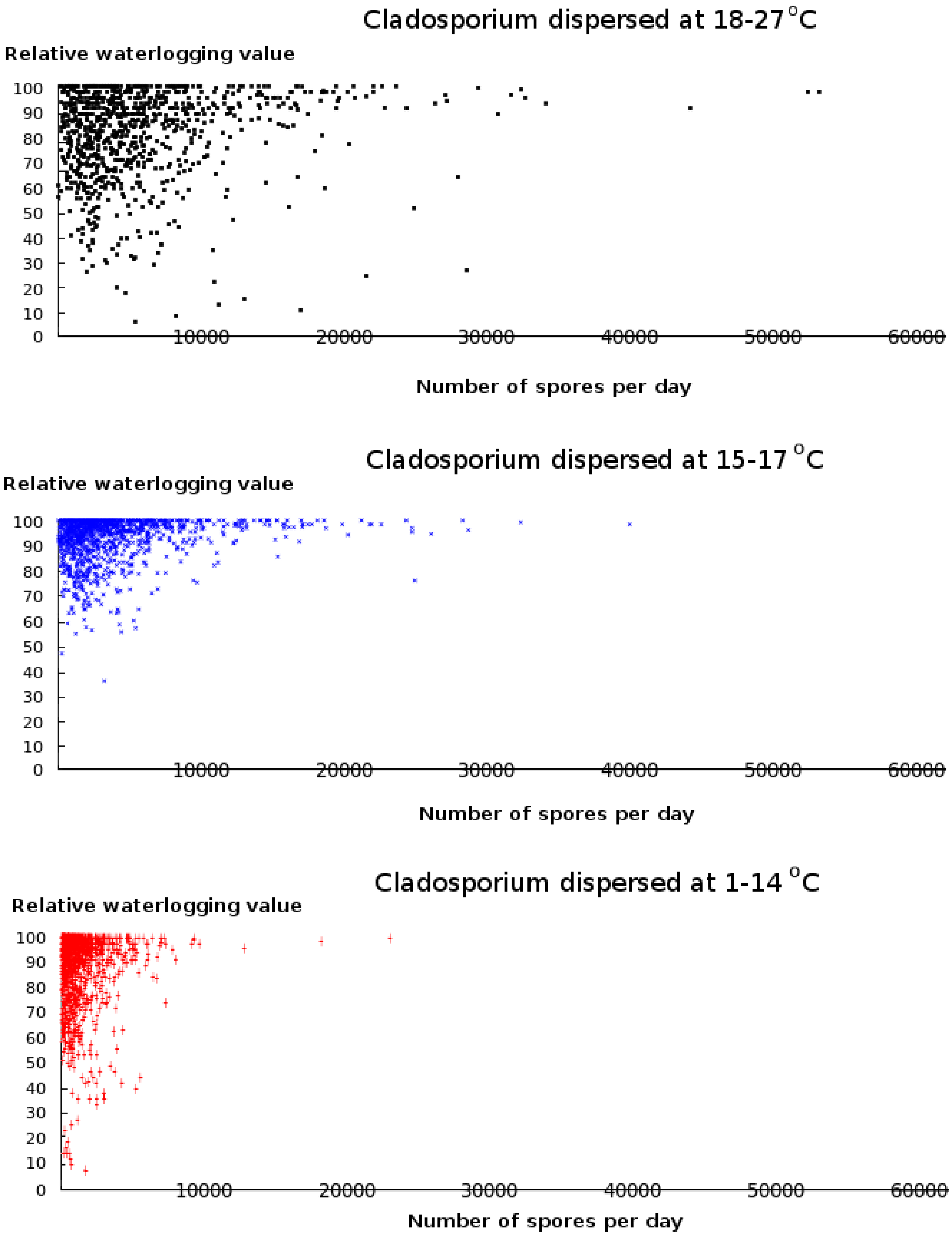


Fig.18. Cladosporium is dispersing its spores at peak-level (above 10000 spores/m3 per day) when the relative waterlogging value is close to 100% and the temperature is 15°C and above.

At high-temperatures the air contains more moisture. That may be the reason why the Cladosporium dispersion seams less dependent on the waterlogging value than at lower temperatures. Beside elaborated by high temperatures, most Cladosporium spores are found, when the soil is fully saturated (high relative waterlogging values).

Indicating high sporification at daily temperatures(Fig.18) (above 14°C), waterlogging (remaining water content after 10mm precipitation) and may be helpful parameters to be aware of to predict high growth and dispersion of Cladosporium spores. However, a locale-scale and multi-parameter (wind, soil-type, surface drain off, sun exposure..) study may clarify the precise thresholds of the most decisive parameters such as the soil temperature, stepwise waterlogging and level of present-day precipitation. These will improve any prediction-model for allergy prevention and farming management of mold fungi.

Beside temperature, other parameters may be of major interest.

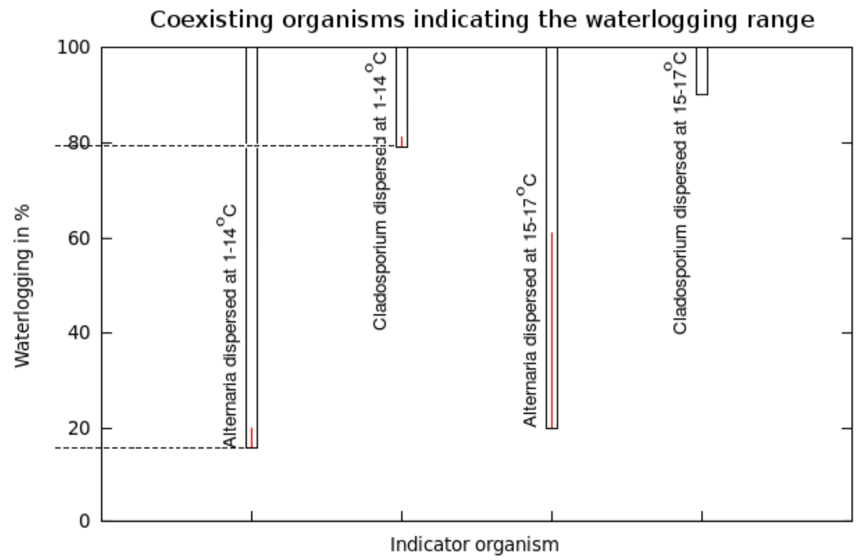


Fig.19. Waterlogging values indicated are too wide for practice usage. However, this parameter may be the best option as relative moisture and barometric pressures at ground-level is only registered during the last 10 years. The principle of indicating waterlogging and with it possible drought conditions, may be of major interest to agriculture management.

Usage of Bio-proxies – to indicate changes in climate and in biodiversity

Changes in phenology that relates to a short reproductive period of the organisms may indicate climate changes (like pollination, Rasmussen, A. 2002). A study of Kinver, M. (2010) found that both a herbarium data-set, which covered a 111-year period from 1848 to 1958, and the more recent field observations showed that for every 1°C increase in the average spring temperature, the orchids flowered six days earlier.

In this study with data from 27 years, peak-dispersion sporification of micro-fungi and fruiting of climate-zone specific macro-fungi are used to find any:

* Changes in the amplitude of organisms within their optimal reproductive phenologic stage
* Changes in start and end time of the reproduction high-season
* Changes in composition & distribution of species in their reproductive phenologic stage

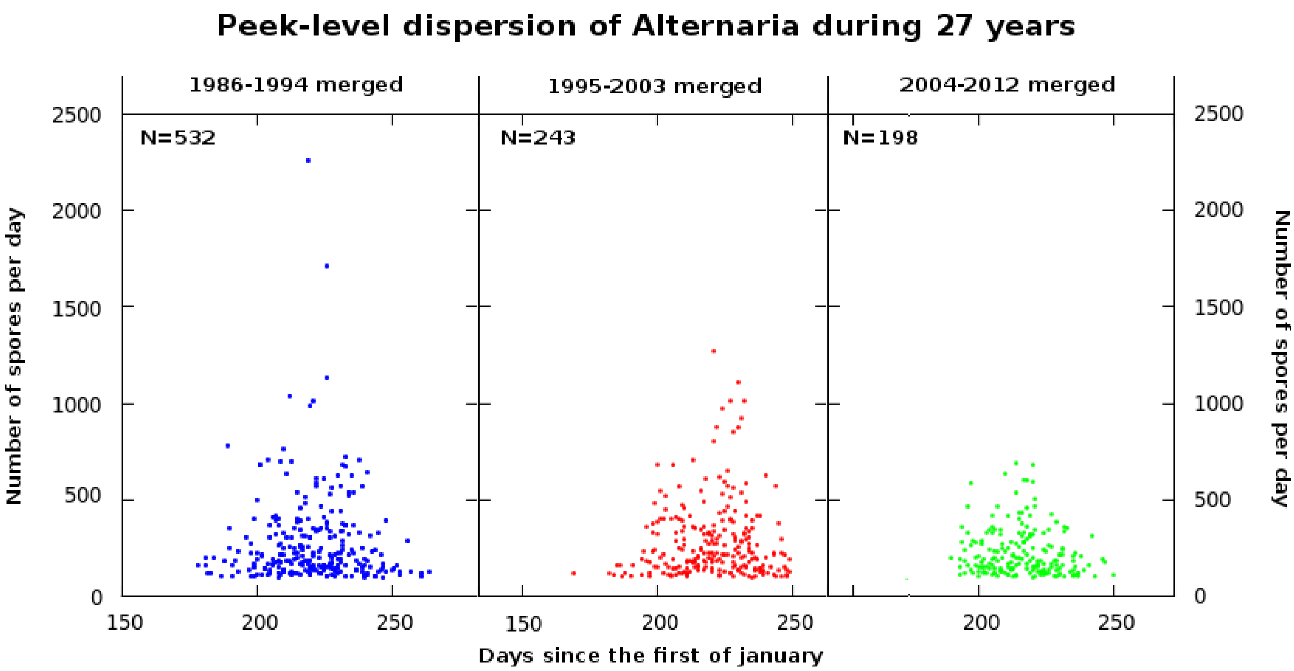


Fig.20. A decrease from 532 to 198 incidents are found of the airborne Alternaria spores at peak-level (the allergenic level above 100 spores/day/m^3 *in 15m altitude*). The dispersion is lessening, so it appear to start later and end earlier. Climate related?: The increased precipitation may have rained-out the spores of the air or less days with 0 precipitation have made dispersion possible during the 27 years(Table B).

As seen in Fig.20, the whole distribution of microfungi seem lessened: This look like a lower

amplitude, a later start and an earlier termination of the peak-situation of Alternaria. The average yearly duration of the peak-level period of *Alternaria* has decreased from 30, to 26, to 22 days through the periods 1986-1994, 1995-2003 and 2004-2012. When any local precipitation is registered in the peak-period, the mean number of *Alternaria* has decreased from 279, to 243, to 207 spores. This accords to the number of incidences at peak-level has decreased from 532, to 243, to 198 in the three periods.
A possible explanation is that dispersion of these exceptional heavy *Alternaria alternata* spores are expected to occur instantly when the 'weather is right': during dry periods in humid regions (Steinman, H. 2011). These observations match the higher precipitation in the late-summer from 1986-2012 of the climate-changes and the number of warm days (>15°C) and days with precipitation as seen in Table B. below.

Temperature and precipitation in the corresponding period (1986-2012)

Table B.

Period	Days with temperature >15°C	% days with temperature >15°C	Days with precipitation (> 0mm)	% days with precipitation	Total days registered
1986-1994	616	49,5	508	40,8	1244
1995-2003	679	54,4	526	42,1	1248
2004-2012	759	67	536	47,3	1133

Table B. As the geophysics has emphasized for the last 30 years, the corresponding number of warm days (>15°C) and days of precipitation (>0 mm) has increased. That is even in this short period of this study. We cannot direct this definitely to climate changes, but the increase may have affected the number of spores that disperse in 15ms' elevation.

Also, occurrence of Alternata dispersion at peak-levels seem to be useful exposing trend of Alternaria alternata for short- to medium term related to incidences of allergenic reactions. Improved local spore-registrations at ground level with and without vegetation may better mirror day to day risk of allergenic weather, especially for children(2).
Knowing the spore-number of Alternata in e.g. stratified soil deposits may expose the levels of middle to long term occurrence. “Unlike pollen from plants and trees, mold spores do not disappear with the first killing frost”(5).

The average duration of periods with peak-level dispersion has decreased from 36, to 23, to 25 days through the periods 1986-1994, 1995-2003 and 2004-2012, respectively. In the peak-level period, the mean number of *Cladosporium* has decreased from 324, to 210, to 226 spores, accordingly. Although, an explosion of airspore dispersion appear to deviate from Alternaria at about 12ºC and Cladosporium at 15ºC, waterlogging may better indicate the climate for the fungi production. An equal number of registrations are selected for the 3 categories (below 15, 15-17 and above 17 °C). Since 1995, a sudden release of more than 10000 spores may appear above 15°C, which may be useful for prognosis. Registering the spores (15m above ground level) there are significant less risk of a spore dispersion when the temperature is low(Fig.21). This seem also true when the precipitation is high. That correspond to a low relative moisture making the spores easier to fly.

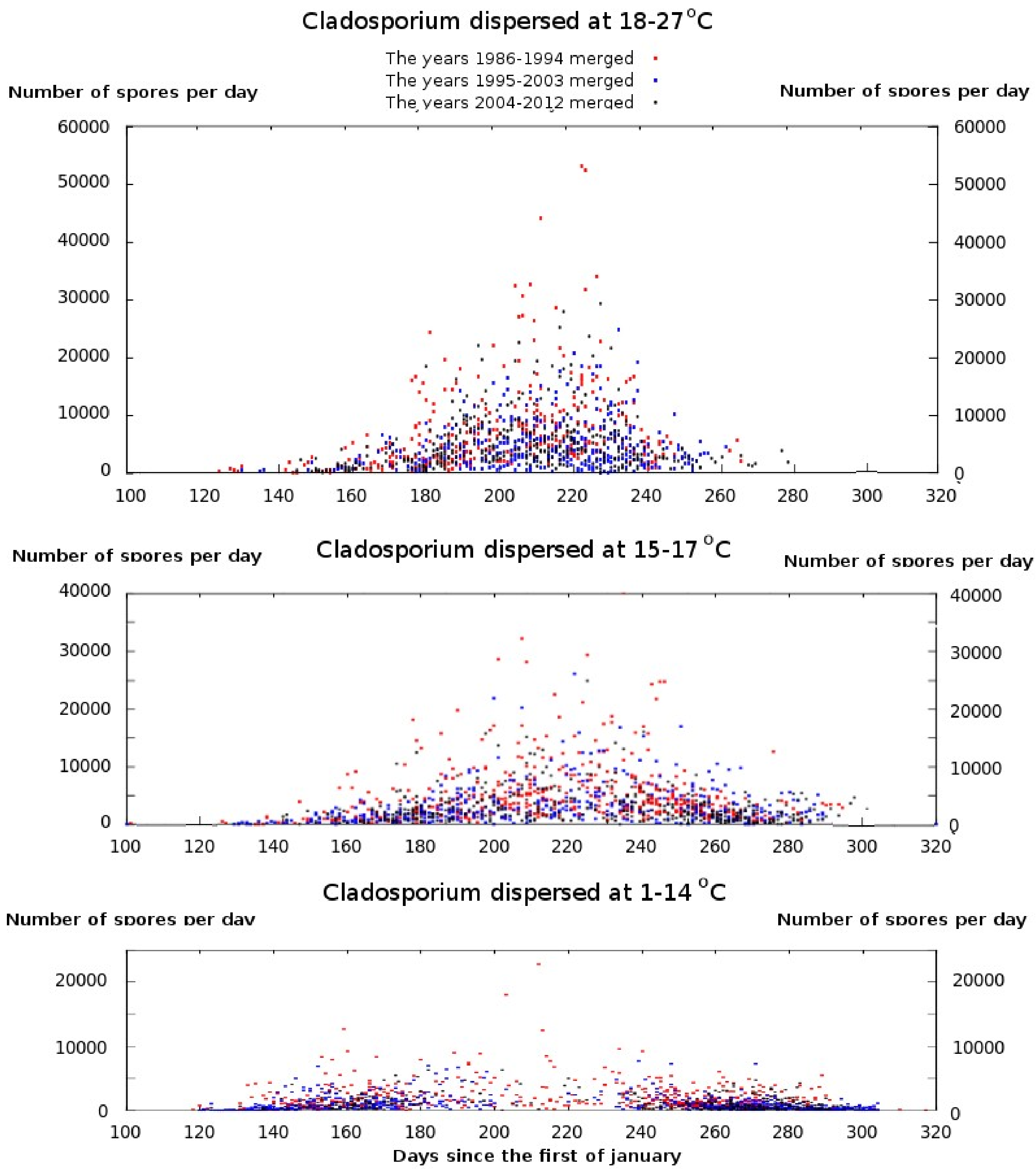


Fig.21. At a threshold above 14.2°C, an explosion-like dispersion of Cladosporium seem to appear. In temperatures below 14°C a few incidents of peak spore dispersion did happen from day 200-235 before 1995, which could reflect a change in temperature tolerance of Cladosporium.

The climate zone of the fungi species

The present status of which climatic zones the fungi are common or are rare may mirror the climatic adaptation for the macrofungi studied. Based on European data (Vesterholt, J., et al. 2008), the Semi-warm adapted Amanita[Table 1] and the warmest-adapted Bolete fungi[Table 2] are still common in the temperate climate zone, while absent or only found occasionally in the Boreal or colder climate zones. This appear reversed for all the cold-adapted macrofungi species of the study.

Amanita classification related to their climate zones.

Climate_category	Species	Temperate	Hemiboreal	Boreal
Cold	A. crocea	rare	common	**common**
Cold	A. submembranacea	occasional	occasional	**common**
Semi-warm	A. pantherina	common	**common**	occational
Semi-warm	A. excelsa = A. spissa	common	**common**	occational
'Warm'	A. phalloides	**common**	occational	N/A
'Warm'	A. junquillea = A. gemmata	**occational**	occational	rare

Table1: Selected Amanita species classified according to their climate-zones (Vesterholt, J., et al. 2008).
cold-adapted are species still common in Boreal climate-zone (CZ): *Amanita crocea + A. submembranacea.*
Semi-warm adapted are species more common in Hemiboreal than Boreal CZ: *Amanita pantherina + A. spissa.*
Warm adapted are species more common in Temperate than Hemiboreal CZ: *Amanita phalloides +A. junquillea.*

As for the Bolete fungi below, it seems possible to separate Amanita to apply to the Köppen climate zone classification based on native vegetation.
It appear possible to assess how their distribution may have changed after the last decades of warming from combined information:
- on these climate categorized fungi,
- on the local temperature & precipitation the last 27 years(table B),
- on their possible role as bio-proxy of these parameters and
- on the present re-finds of the fungi locally in the temperate zone near Copenhagen.

The semi-warm fungi appear temperature-indifferent and may have become the most common species, locally.

Bolete classification related to their climate zones.

Climate_category	Species	Temperate	Hemiboreal	Boreal	Subarctic/alpine
Cold and indifferent species	Leccinum versipelle (Fr. & Hök) Snell	common	very common	very common	very common
Cold and indifferent species	Leccinum scabrum (Bull.) Gray	very common	very common	very common	very common
Cold and indifferent species	Suillus bovinus (Pers.) Kuntze	very common	very common	very common	very common
Cold and indifferent species	Suillus luteus (L.) Gray	very common	very common	very common	very common
Cold and indifferent species	Suillus variegatus (Sw.) Kuntze	very common	very common	very common	very common
Cold and indifferent species	Leccinum holopus (Rostk.) Watling	occasional	common	common	common
Semi-warm	Leccinum variicolor Watling	common	common	common	occasional
Semi-warm	Suillus grevillei var. grevillei	common	common	common	occasional
Semi-warm	Boletus edulis Bull.	common	common	common	rare
Semi-warm	Chalciporus piperatus (Bull.) Bataille	common	common	common	rare
Semi-warm	Tylopilus felleus (Bull.) P. Karst.	common	common	common	
Semi-warm	Suillus granulatus (L.) Snell	occasional	occasional	occasional	rare
warm	Boletus badius Pers.	very common	very common	common	
warm	Boletus rubellus Krombh.	occasional	occasional	rare	
warm	Boletus calopus Fr.	occasional	occasional	rare	
warm	Suillus viscidus (L.) Fr.	occasional	occasional	rare	
warm	Suillus cavipes (Opat.) A.H. Sm. & Thiers	occasional	occasional	rare	
warm	Leccinum duriusculum (Schulzer) Singer	rare	rare	rare	
warm	Boletus impolitus Fr.	rare	rare		
warm	Boletus parasiticus Bull.	rare	rare		
warm	Boletus pulverulentus Opat.	rare	rare		
warm	Boletus radicans Rostk.	rare	rare		
warm	Boletus appendiculatus (Pers.) Krombh.	rare	rare		
warm	Boletus satanas Rostk.	rare	rare		
Warmest	Boletus pruinatus Fr. & Hök	very common	common	occasional	
Warmest	Boletus chrysenteron Bull.	common	occasional	occasional	
Warmest	Boletus luridiformis Rostk.	common	occasional	rare	
Warmest	Boletus porosporus Imler ex Bon & G. Moreno	common	occasional	rare	
Warmest	Boletus reticulatus Schaeff.	common	occasional		
Warmest	Boletus luridus Schaeff.	occasional	rare		

Table 2. Selected Bolete species classified according to their climate zones (Vesterholt, J., et al. 2008).
Cold-adapted and indifferent are species still common in the subarctic zone.
Semi-warm adapted are species more common in boreal than in the subarctic zone.
Warm adapted are species more common in hemiboreal than in the boreal zone .
warmest-adapted are species more common in temperate than in the hemiboreal zone.

As the Cold adapted Bolete fungi are common in all the shown climate zones, they are denoted 'Cold and indifferent species'. Although they are very common in all European regions, combining the above information described may tell us that these fungi are precipitation-sensitive species and for that reason, they are not found so frequently anymore, in the studied local area.

Discussion

This screening study is outlining *methods* and examples that present what to be aware of to calibrate bio-indicators into bioclimatic proxies and their possible values for indicating climate conditions. The outcome of the methods described are those that actually seem to achieve useful bio-proxies - to workup proxy-data, to deduce changes in climate and to find how the changes may affect the organisms - of relevance for both basic and applied sciences.

Bio-proxies to workup local and time-specific climate data

The criteria of a well-calibrated bio-proxy

Bio-indicators need to be in a defined phenologic stage and to be highly sensitive to their surrounding climate to properly workup climate data. First, the corresponding climate parameters need to be well-defined. Due to improving technology, both weather stations and reconstructed weather models offer data of smaller and smaller grid size. To gather a uniform statistic acceptable number of the fungi, this study had to gather the finest common grid size of the 9 years period.

Why select sensitive fungi that may be extremely diverse according to:
"Seasonality and year-to-year annual variation. A particular species may fruit at different seasons across wide geographic distances or along strong elevational gradients" and "fruiting is influenced by elevation and latitude and their effects on temperature and precipitation” (Lodge D.J. 2004).
"Fruit body appearance depends on temperature, precipitation and its timing, soil pH and nutrient availability, as well as successional patterns in the vegetation”(Gates G.M. 2009), and
"Fruit body production must be linked with the growth of the associated host trees” (Egli. S. 2010).
This immense variation is also found in this study[appendix X]. However, the point is that fungi appear sensitive to their 'perceived' (micro)climate: Already in 1994, Smith and Theirs suspected suitable microclimate conditions of Bolete fungi to be:

"Particularly humidity, soil moisture, and soil temperature necessary"and Gates G.M. (2009) found “Using temperature and rainfall data, the presence of *fruit bodies* is seasonal but not directly attributable to rainfall events”. For most fungi species, combinations of many unsolved parameters appear decisive. But for a few fungi the variations in this study seem to be in restricted ranges for single decisive climate parameters: daily temperature, precipitation (Poonam Sharma, 2004) or a well-definable mixture of parameters such as prior precipitation(waterlogging). The indicators appear liable, when the organisms are selected according to a specified phenology. In this study, it is when sporifying fungi of selected genera are peak-emitting (above 100 for Alternaria or 10.000 for Cladosporium), or mycorrhizal fungi adapted to similar climate-*zones* (e.g. warm adapted Amanita).

Besides, fungi are not nescessarily seasonal: “Unlike pollen, there is no sharp seasonal variation in the atmospheric fungal air-spora. However, some variations occur due to extreme weather factors” (Gitay, H. et al. 2002).
*"*Cladosporium is the leading allergenic mold in cooler climates” (Cramer, W. 2002). So is temperature the solely decisive parameter that favor Cladosporium to peak?: Spore numbers (of Cladosporium) in the air are increased dramatically as a result of agricultural practices such as harvesting of cereal crops or making hay (Celenk, S. et al. 2007). "Seasonal and yearly variations were shown by the spores in relation to local agriculture" of Alternaria and Cladosporium in England (Poonam Sharma, 2004). Theoretically, harvest could be a secondary effect of the climate and could affect an even enhanced level of the peak-level. However, we find (in 15m's altitude) a restricted time of peak-levels through the 27 years and the amplitude of the whole distribution has lessened. If harvest was decisive in 15ms altitude, it would only make the "peak season" arrive

earlier as for orchids (Kinver, M. 2010) and for pollen (Rasmussen, A. 2002). Besides, it would not decrease the peak-levels throughout the years. In fact, we find the allergenic fungi to peak *later* - as for the macro-fungi: “Autumnal fruiting date of mushrooms has delayed in fruiting since 1980 of 12.9 days in Norway over the period 1940–2006” (Kauserud, H. et al. 2008).

It is not the ambition of this study to claim that either rainfall or temperature "could separately explain conclusively the variation in fruiting body emergence of macrofungi fruiting", and "it is likely that temperature has an indirect dry out effect” (Gates G.M. 2009) preventing fast decay. Even though temperature is found almost pure decisive for some fungi, it still remains uncertain, why some fungi are able to indicate local and very time specific climate-data. But for the proxy purposes, the 'why' is less important. This suggest that the need of 'probability indication' will be the most liable indication method. Different reliable climate-data can be worked-up from different bio-proxies:

Indicating temperature data

No single parameter can be *proven* 100% decisive for distribution of any of the selected species: Temperature does not *imply* a certain number of spores to disperse from e.g. Alternaria, nor does temperature alone *imply* fruiting of species to grow - like Amanita.
In a Japanese review paper, Yamashita, S. & Hijii, N. (2004) round up that "no significant relationship between any factors of temperature and the number of fruit bodies in any dominant genera of Amanita". Similarly for this study, a certain temperature does not imply any development of any of the fungi studied, either. *It is the other way around*, if the *selected* fungi are present in a certain development stage, they seem to indicate a delimited temperature range.

The found indicators are:
--> *microfungi above a certain threshold values* and of *a specific phenologic sporification stage*
--> *macrofungi from bioclimatic groups of specific climate zones are* selected taking into account the *development time* of their *fruit bodies*.
Some of the chosen fungi. However, it is only when Alternaria and Cladosporium are found to indicate temperature at the allergenic peak-level and of a specific sporification stage that the temperature appears to be decisive. That is, if the number is found to exceed 100 Alternaria or 10,000 Cladosporium per m^3/day. "The atmospheric concentration of Alternaria spores are markedly affected by meteorological factors such as air temperatures and barometric pressures.” (Kilic, M. 2010).

Temperature is also regarded as "the best predictor for Alternaria and Cladosporium fungi" at 3m's altitude (Poonam Sharma, 2004). Another pick is merged Amanita and Bolete fungi-species of bioclimatic groups from specific climate zones(Table 1,2) taking into account the development time of their fruit body (3 days). The classification method for bioclimatic grouping is carried out to compare the local composition of species with that general classified at different climatic zones. Besides, it was selected to avoid a tautology, which would be the case using parameter-classification (del Río, S. 2007).
An important goal is to find reliable and narrow temperature ranges of their environment by using multiple bio-proxies for especially the 'probability indication' method.

Finally, the present study exposes each year of frost by the last appearance of these frost-sensitive fungi-species, as the fruit body of mycorrhiza fungi instantly respond to 'perceived' exposure to frost in their (micro)climate.

Indicating precipitation data

"Temperature and water availability are key factors for the fruit body formation of forest mushrooms. It is a widespread experience of mushroom pickers that drought periods inhibit or delay fruit body formation” (Egli. S. 2010). "Although the fruiting of macrofungi most often is limited by lack of precipitation, excess moisture also can prevent fruiting in some species” (Yamashita, S. &Hijii, N. 2004). In the present study, this depends on the group of fungi. For the dispersion of the micro-fungi, it seems likely that precipitation takes over from temperature being the decisive parameter when the precipitation is above 20mm. It may be due to lessening numbers of 0-dispersion days or the small Cladosporium may rain out of the air, while pollen and Alternaria remain until higher precipitation. This miner difference may be used for indication of the extreme rare incidents of precipitation above 23mm precipitation.

For the semi-warm adapted Amanita and the warmest-adapted Bolete fungi, temperature and not precipitation appear decisive. In contrast the *cold-adapted* Amanita and Bolete fungi seem essentially about *low precipitation*. The other fungi seem influenced by a mixture of different climate parameters or by climate remote parameters. "Above average rainfall may result in the rapid presence of large numbers of boletes, in what is known in some circles as a 'bolete year'” (Eduard, W. 2009). This classic dependence of high precipitation, we could not appoint to any *specialized* group of fungi, but abundance of fruits of the temperature-indifferent fungi are found in the summer, too. Many individuals are found of the indifferent and semi-warm adapted Amanita and warmest-adapted Bolete fungi, when the precipitation is high, but many of these also fruit at lower precipitations. To indicate high numbers of fungi to high precipitation, we need a numeric data-set that specify the numbers found in each location.

Indicating a combination of climate parameters

Is it possible to define a indicator based on combinations of indicators? In principle, it is possible if a combination of e.g. temperature and precipitation is consistently decisive for the organism. It could be the most narrow matrix of temperature and precipitation range from 11-19°C and 0-6mm being the most probable for the warm-adapted Amanita(Fig.6). They may be used for absence indication as they are rare or absent below 11°C and when precipitation is present. However it seem difficult to assess if other parameters may be decisive as they are absent at very low, at very high temperatures and at high precipitation at summers before day 200(Fig.7).

Waterlogging data (influence of preceding precipitation and draying out)

For some possible indicators, it may be more liable to define a functional parameter that is a fixed combination of linked decisive parameters. A defined 'waterlogging value' could describe the remaining water available for some fungi. Idealistically this value should cover time since the last precipitation, drain off due to slope and soil type, wind, evapo-transpiration due to temperature, sun, albedo, shelter etc. As the sample area of this study is homogeneous, the 'waterlogging value' used is a rough simplification. It includes only precipitation, days since last precipitation, temperature and a constant (for dry out). The simplicity may be the reason for the huge variation. The results for Alternaria and Cladosporium may describe some remaining interpretation of the climate ONLY when the major decisive parameter is excluded (by splitting up in temperatures of the found categories of Cladosporium 1-14, 15-17 and 18-27°C seen at Fig.21). The 'success' of this parameter may be appointed to the antipole of extreme drought (>10 days of no summer precipitation) or completely soaked soils that is waterlogged (>10 mm precipitation).

Usage of Bio-proxies – to indicate changes in climate and in biodiversity

Access changes in climate and biodiversity based on how key organisms (of sectors) have changed the past 27 years with increasing temperature and precipitation.

The method used in this study can be split into *Adjustments, Calibrations* and *Indications*. These are straight forward to be expanded depending on the usage, but an overall method that may cover most usage areas would be:

*Bioclimatic mapping method

- *Adjustments* of biologic and physic data e.g. adjust older 'adjacent weather-parameters' to satellite data or reconstructed data into fine-grid data at ground level.
- *Calibrations:* Find which adjusted parameter(s) that is decisive for the potential indicator by correlating present fungi data with the decisive adjusted data. That way, ell-calibrated indicators become bio-proxies.
- *Indications:* Refinds of bio-proxies to indicate the climate at a specific locality and time.
- *Control:* Verify climate model-data against the observed and bio-indicated climate-data using reverse CO_2-equivalents of the temperature indicated.
- *Databasing:* Add verified data into e.g. the Fine-Scale Road Stretch Forecasting - to fit outside roads by 'optimal interpolation' of the controlled data.
- *Bioclimatic mapping:* Add all available coherent data into Overlay mapping (GIS) - to produce local-detailed Bioclimatic maps(like Fig.31, but including soil maps, topography, land-use etc.)
- *Assess the migration & dispersion:* evaluate changes from map to map - in time and space
- *Public & applied tool:* produce a GPS-based app. for smartphones.
- *Contribute new data:* When no more verified bio-datasets are available, citizen-data are included (the Wikipedia or Danish fungi-atlas way of Heilmann-Clausen, J. et al. 2013) into new *Adjustments* ^.

Working-up climate data of the past from bio-proxies

A point is climate indications back in time within specific locations and in a high temporal resolution of even hours. This is a screening for guidelines to find *living bio-proxies* that point back in time. That is, indicators that respond to immediate microclimate 'snapshots' when the indicators were present in a location – or when the composition of species has changed mirroring longer term climate changes. First, finds of living bio-proxies gain that these are testable. Beside the peak-level responding *Alternaria alternate* and *Cladosporium herbarum*, macrofungi connected to their climate zone affinity are found liable.

The group of the semi-warm Amanita fungi seem to be temperature indicator, and so may its involved species (*A.pantharina* and *A.spissa*), too. The same may apply to the 7 warmest-adapted Boletus fungi even though *Boletus pruinatus* deviates from the other 'sommer Bolete' fungi, as it has optimum in October (Heilmann-Clausen, J. et al. 2013). Alternaria and Cladosporium dispersion do not in general indicate high temperatures, but peak-levels of these do appear to have a lower temperature-limit. It appear probable that the Alternaria and Cladosporium distribution are lessening in the 27 years period *according to the climate.*

The present status of the semi-warm adapted Amanita & warmest-adapted Bolete fungi that are common in the temperate climate zone compared to the more rare occurrence of the cold-adapted macrofungi may indicate the already established climate-changes. The cold-adapted are all very common in subarctic and alpine climate zone; they seem negative responding to high precipitation, which has already increased the last 27 years(Table B). From the study the Semi-warm adapted Amanita and the warmest-adapted Bolete fungi are found at any precipitation conditions and without an upper (everyday) limit of temperature. These *temperature-independent*, generalists, seem well adapted to the warmer and more wet conditions of the 27 years of this study, and so they could outmatch the other mycorrizal fungi. The liable change of composition of macrofungi from cold-adapted, warm-adapted to the temperature-indifferent fungi indicate that the composition of fungi (and biodiversity) may be affected by their climate. That is supported by the autumnal fruiting date of mushrooms has delayed in fruiting since 1980 of 12.9 days in Norway over the period 1940–2006 (Kauserud, H. et al. 2008).

Beside climate induced change, culture induced changes of the composition of species may be found: Re-findings of spores, (like pollen) and microorganisms may indicate the temperature and humidity of both natural surroundings soil usage for the people that lived in the locations: Alternaria spores is associated with the grass-family (like cereals) so they indicate an opening of the land - like agriculture, while the Amanita and Bolete mycorrhiza fungi are bound to trees indicating forest conditions. It supports that proxies may reveal "historical and with caution palaeoclimatic processes" (Charles C. et al. 2010). That is "from intraseasonal to intermillennial and it may be analytically-determined or archive-limited" (Lawrence, KS. 2006), and the fungi spores are especially persistent. Spores of *all* the studied fungi are recognizable in protected soils and sediments, in ice cores, in rosin, in fossils etc.

According to Wartchow, F. (2009), Fungi are old 542–488.3 Ma, and mycorrizal macrofungi could, but rarely do adapt, like Aminata or Bolete fungi - in contrast to fast multiplying microfungi without hosts. "Fungi with low host specificities could readily adapt to new hosts" (Feng B. et al. 2012).
A bonus is that the microfungi themselves (sporocarps) and their fast produced spores will indicate the same temperature range. The easy disintegrable fruitbodies of the macro-fungi also coincides with their archiveable 'hard-shell' spores as these are dispersed from the fruitbodies. So it is liable that all spores and sporocarps are equal good bio-proxies, and indicate their surroundings all the way back to their early findings (in an archeologic and palaeontologic timespan). Although, remains of bacteria, such as the biomarkers "TEX86 and MBT/CBT are shown to have a limited applicability in predicting the lake temperature" (Jones, E.P. 2012), it is possible to go backwards using some biochemical traces (Jones, E.P. 2012; Carlisle, A.B. et al. 2012) and DNA.

Comparison study of how to select and utilize bio-proxies

Access how different bio-proxies and methods may indicate different climate parameters and ranges

Keeping the archiving in mind, it is possible to compare the usage of different types of bio-proxies. Hard-shelled organisms like insect-capsula of kitin, incysting microorganisms and fungi spores are often well-conserved. However, they need to be found in a peak-level to mirror local dispersions that reflect local climate parameter. A few of the fruit-bodies of the macrofungi are found suitable as bio-proxies, but they are not well-conserved while their spores are.

The finds of the study confirm the literature that reports that microclimate determines the phenology limits of the organisms (Rasmussen, A. 2002). A few plausible indicators (6 out of 39 studied) do seem to indicate temperature or precipitation, as the fungi expose their 'perceived' climate conditions at local spots (microclimates): The Alternaria alternata, the Cladosporium herbarum, and especially the semi-warm Amanita and the warmest-adapted Bolete fungi may be considered as temperature indicators. In the other hand, the Cold-adapted Amanita and Bolete fungi appear just as useful precipitation indicators. They are, however, too few to workup a narrow range of parameter-data for the climate models. Nevertheless, the *found procedures* and the *principle of multi-proxy reconstruction* appear promising as soon as sufficient bio-proxies are available and when the bio-proxies are more precisely calibrated. If the found bio-proxies tolerances are verified in different locations of their climate zones, these 6 should contribute to a bio-proxy database for temperature and precipitation reconstruction.

Internal verification and working up narrow climate parameter ranges: The co-existing Cladosporium and Alternaria tolerances are found too similar to narrow down any temperature range. The same pattern seem evident for 'co-existing indication' of semiwarm-adapted Amanita fungi (strictly existed at 4-22°C) and the warmest-adapted Bolete fungi (at 3-22°C). However, these two pairs of almost coincident indicators support each others indication of e.g. temperature. That is useful *for control* to discover if species mutate and change phenology: If one of these genus would suddenly adapt (e.g. mutate), the new adapted one would no longer support the other indications.

Organisms do not only adapt genetically but also phenologically in competition with other organisms. E.g. if a competing fungi extinct, the indicator may live in a wider temperature range than found during the calibration. Coincident indicators act as a control procedure to prevent *in vivo* "overestimation of climate changes” (Ashcroft, M.B. 2010). Furthermore, non-climatic adaptation is rarely happening for mycorrhizal fungi due to their co-adaptation to the trees.

The studied *mycorrhizal* fungi support each other and so do the *statistically* well-founded microfungi. The fungi are common and are not likely going to extinct or change genetically precisely the same time. To such coincidence to occur, the habitual and genetical makeup of the very different indicators found are too wide. Besides, grouping like the 'cold-adapted', 'semi-warm adapted' and 'warm-adapted' species(Table 1,2) should minimize such risk.

Finally, the high number dispersion of microfungi (spores) provide a very good statistic foundation and are, therefore, liable for 'absence indications'. To narrow down ranges of climate parameters, *different organisms (taxa) eliminate temperature ranges* as the allergenic micro-fungi and the mycorrhiza fungi do when they coexist. That way it is illustrated that 'co-existence indication' of very different taxa seem to provide a method to indicate more narrow climate range. In some areas, only few calibrated indicators may be refound. Few well-calibrated indicators may indicate a probable narrow climate range using 'probability indication' (illustrated in Fig.12). And when the bioproxies have turned out to be consistently indicating the same distribution - as the allergenic fungi that vary by date, but not by temperature minimum - the 'probability indication' may effectively reduce the number of needed indicators to be calibrated.

The larger number of well-calibrated bio-proxies that are re-found at the same location and time, the better they delimit a narrow range such as ±0.1°C and ±0.2mm precipitation. Multiple bio-proxies in a database can verify or offer new data for climate models. Indicated temperatures may add CO_2 equivalent data into independent bio-models and take into account local footprints into the final scenarios - like the geophysics do in their global climate model (Shuting Yong 2012).

Choice of key-organisms of sectors – to provide applied science results

From key-organisms of sectors the upper indicated climate results are provided for basic science, but sector information is achieved during the same procedure.

Below, examples are given of direct or indirect interactions between the sectors, their key organisms and changes in climate parameters.

- Healthcare of Allergy

Due to the need of local dispersion and good statistical foundation, the peak-level dispersion of allergenic fungi spores were an obvious choice. Besides, answers about allergic levels of spores may come forward as the peak-level of this study are set to the medical morning PEFR allergen level – a so high level that the probability of outbreak is close to certainty among sufferer that are allergic to these fungi. The spore dispersion of Alternaria and Cladosporium into the air do appear to decrease during the last 27 years in summer-autumn *in 15m.s' altitude*. It coincides with the less dry days where the spores disperse (or the spores are raining out).
As the peak-level of spores clearly increase when the temperature and after the autumn rain increase, so must the allergenic spores where they are produced – which is at ground level. That correspond to "A recent study found that at ground-level (50 cm), Alternaria spore concentrations are significantly higher in the presence of vegetation, suggesting that the individual's exposure to Alternaria, especially in the case of children, is underestimated by samples taken at roof-top level by a fixed volumetric collector” (Ai-ping Zeng, 2011). When the recurrent dispersion above critical peak-levels is verified to be correlated with the climate, any increased warm and moist conditions at ground level is bad news.

- Forestry and Agriculture

The typical forest species, Amanita and Bolete fungi studied, appear to shift from specialist species to indifferent species according to *precipitation*, as described above. The cold adapted Bolete fungi according are more indifferent to temperature and common than the warmest-adapted species(Table 2). The semi-warm Amanita and the warmest Bolete fungi that appear to be precipitation-indifferent seem to be species that are also more frequent mycorrhiza species of deciduous trees compared with those associated to conifer species: Only Boletus pruinatus, B. chrysenteron and Amanita excelsa are occasionally found under conifers. All other semi-warm Aminata and the warmest-adapted Bolete fungi are typical under deciduous trees. If verified in the Oslo area, it may be the reason why the population of spruce was needed to be cut down - a possible consequence to weak nutrient uptake and small root-area without adequate conifer-mycorrhiza.

The relation to climate data that is found for Alternaria alternata is liable true for the other mold-species of Alternaria (*A. solanis*) and probably other microfungi. That is despite A.solanis may disperse differently: As neither Fusarium or Alternata solanis is found in noticeable amounts in the volumetric spore trap at 15m, they do not disperse far from their sources. Besides, instead of relating everything to unmanageable seasonality, any close climate relations of microfungi can make warnings possible and help choosing crop species that depend on local climates.
As described about the species of the Amanita and Bolete fungi, the found change of composition from specialist to precipitation indifferent species, is liable for the agriculture species, too. Our found change in composition is supported by the literature that also describe shifts from specialist species to generalist species of microorganisms (Yergeau.E. et all. 2011). Mycorrhiza is well-known among agricultural crops, but these microfungi may even faster change composition due to their very fast reproduction cycle. Not all microfungi are benign or beneficial mycorrhizal fungi, the pests may change according to the climate, as well. And their natural enemies are always delayed when they co-adapt. Again the consequence may be to reconsider the choice of crops to generalists and reflect weather and climate changes. They may better match high temperature and precipitation, extreme weather incidents or stand the pressure of new pests.

- Conservation and Landscape management

For conservation and landscape, the 2 most interesting finds of the study are the classification of
→ indicator species with the same tolerances as endangered ones. Besides, the indicators may directly reflect their micro-climate of their very local area. That is valuable to categorize:
→ suitable (cold) areas to adapt due to their bio-indicators. Although, a few cold-adapted Amanita and Bolete fungi are found, the locality studied are populated with semi-warm Amanita and the warmest-adapted Bolete species that tolerate heat. Being close to the capital, the location may be warmer, and the area may be invaluable for cold-adapted species: The precipitation in the area has also increased the last 27 years and for that these have a low tolerance. That way the livelihood of each local areas may successively be clarified for survival (and inserted into Bioclimatic maps*).

- Public climate- and nature-awareness

The location studied is a common area for collection of eatable fungi. Whenever predictions of climate-changes are liberated, such as increased precipitation and heat in the Nothern hemisphere, the warmest-adapted fungi (like the indigo-colored Boletus) are expected to expand on behalf of the cold-adapted ones. The common poisonous Amanita fungi have generalists that may also profit of the future climate: The Semi-warm adapted - such as Amanita pantherina - and the Indifferent Amanita species may be more frequent on behalf of the specialists species (cold or warm adapted ones). However, climate related predictions are difficult, but weather-predictions are liable and will bring attention to the collectors. Including more indicator-organisms will increase public awareness.

Whereas the present study was time limited, all the sector issues mentioned would be elaborated in the “Perspectives and further to the study” paragraph in the Appendix including the main method as part of the "How fungi disperse and migrate” project. That is to study climate-sector processes by follow their microclimate in their place of origin, their air route and where the fungi settle using the *Bioclimatic mapping method.
This method appear useful to deliver maps that visualize how the climate is developing from time to

time and through the landscape. If we implement sequences of the Bioclimatic maps and assess how 'dispersion and migration' of the organisms proceed, the succesive movements of the species can be followed. When the members of the sectors themselves can find more local and timely information from the Bioclimatic Maps, the sector consultants will be able to advise on a more informed basis. Volunteer authorities and the consultants can assess and contribute new information into the Maps.

Users of Bioclimatic mapping will be able to follow

- *bioclimatic indicators*
- *new crops, invasive species and pests*
- *allergenic agents*
- *native species*

through time and location - using Bioclimatic mapping will motivate

- *scientists*
- *farmers and forest managers*
- *patients*
- *managers of biodiversity resources for recreation, gene-banks and bionic inventors and the general public*

to look into changes indicating climate and from this biologic interactions.

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Appendix.

Robustness and elaborating the figures shown

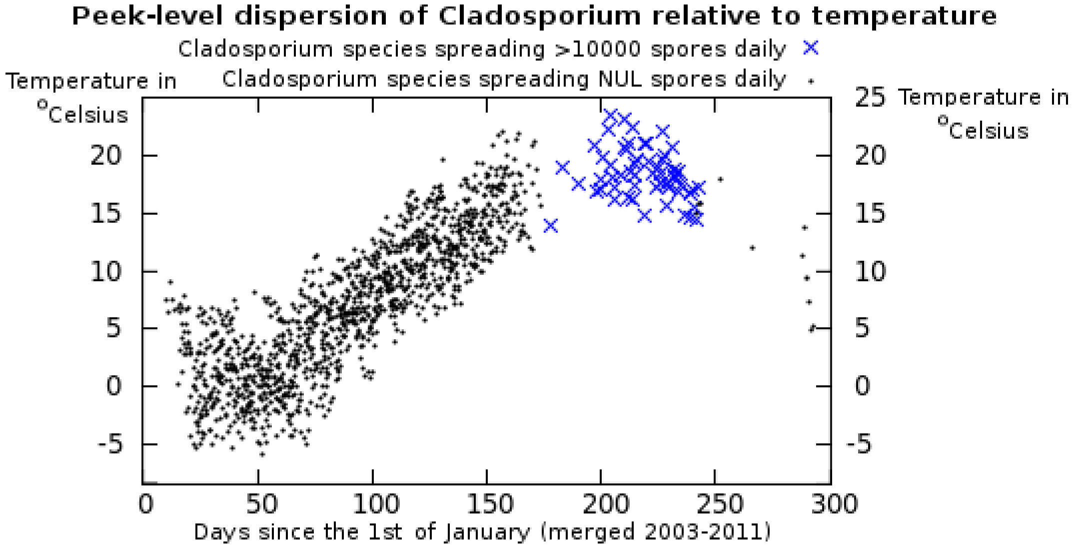


Fig.22. The indication of precipitation is, like that of temperature, not restricted to a certain period. If the climate parameters are right (no precipitation) we'll find the spores of these indicators. Abundance and time-independency make them more useful. Cladosporium at peak-level is found above 13°C.

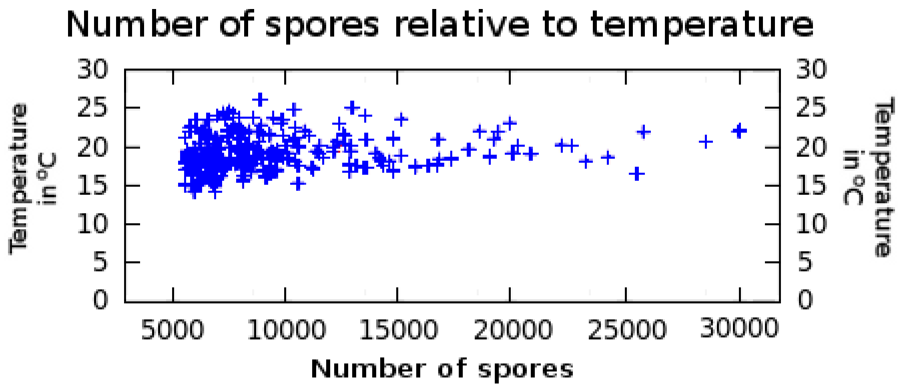


Fig.23. The Cladosporium spores at peak-level (above 10,000 spores m-3day-1) are found above 14.2°C (and between 5000-9999 no peak-level below 12.3°C) in the calibration period (2004-2012).

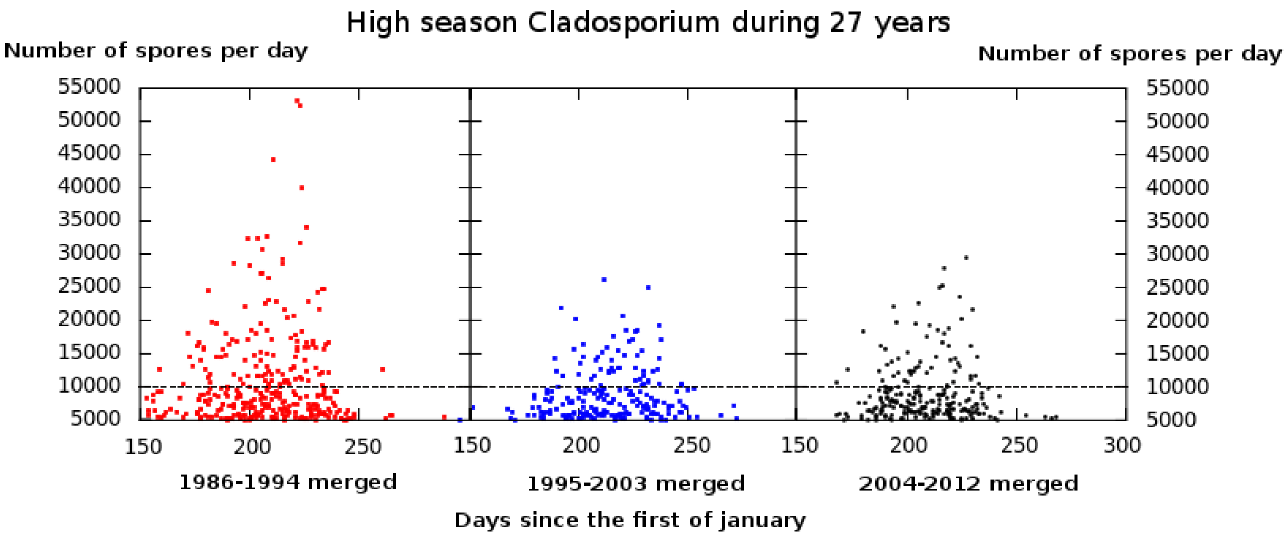


Fig.24. From 1995-2003 to 2004-2012, any climatic change of Cladosporium is less clear as Alternaria(fig. 20). The Cladosporium spores are significantly smaller than Alternaria.

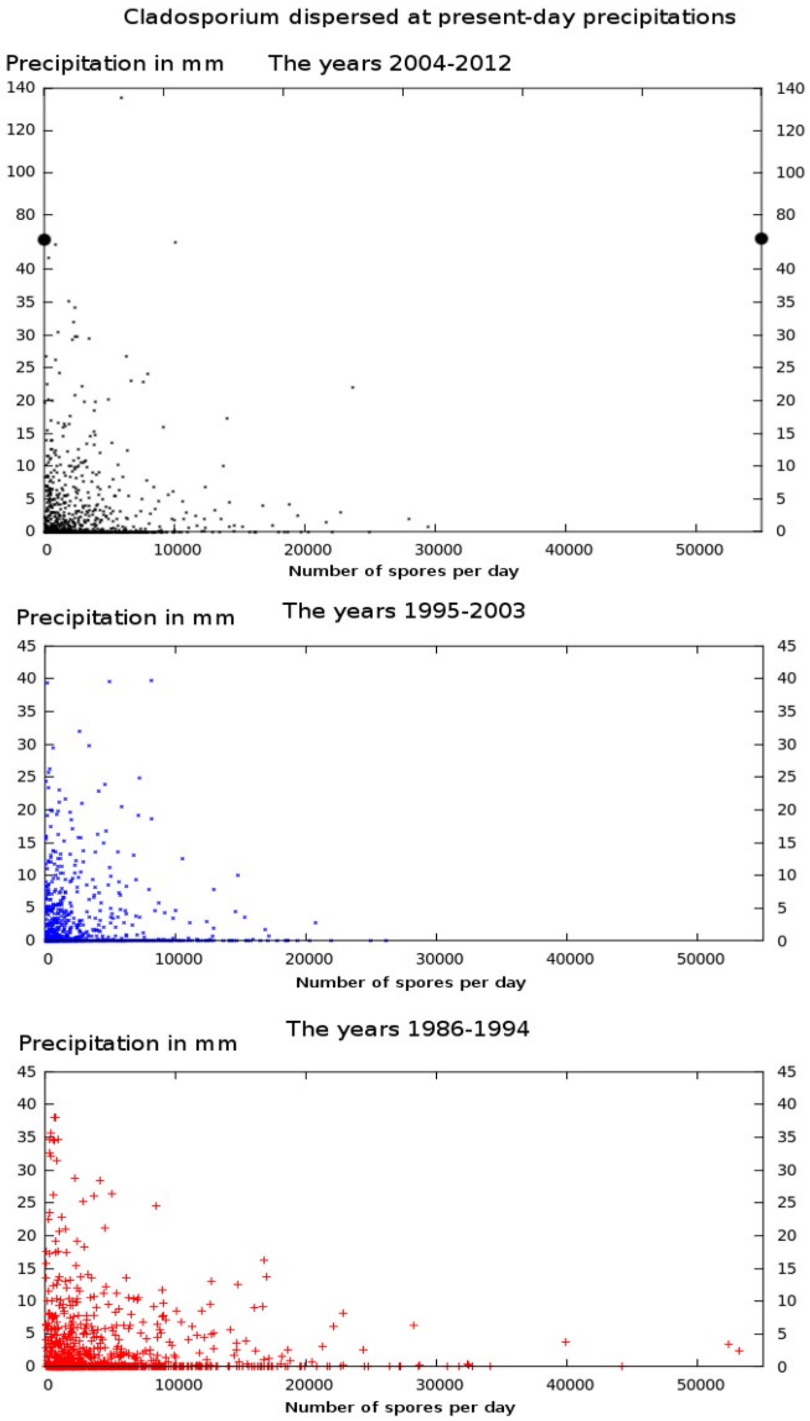


Fig.25. The peak-dispersion of Cladosporium reaches extreme high levels only within a restricted period. The change in the peak-period above 10.000 spores provides the same pattern as that above 5000 spores/day/m^3. As for Alternaria, the number of dispersing spores decreased from the range 1986-1994 and till 1995-2003. It is not evident in the latest period. The starting time seem to set in later.

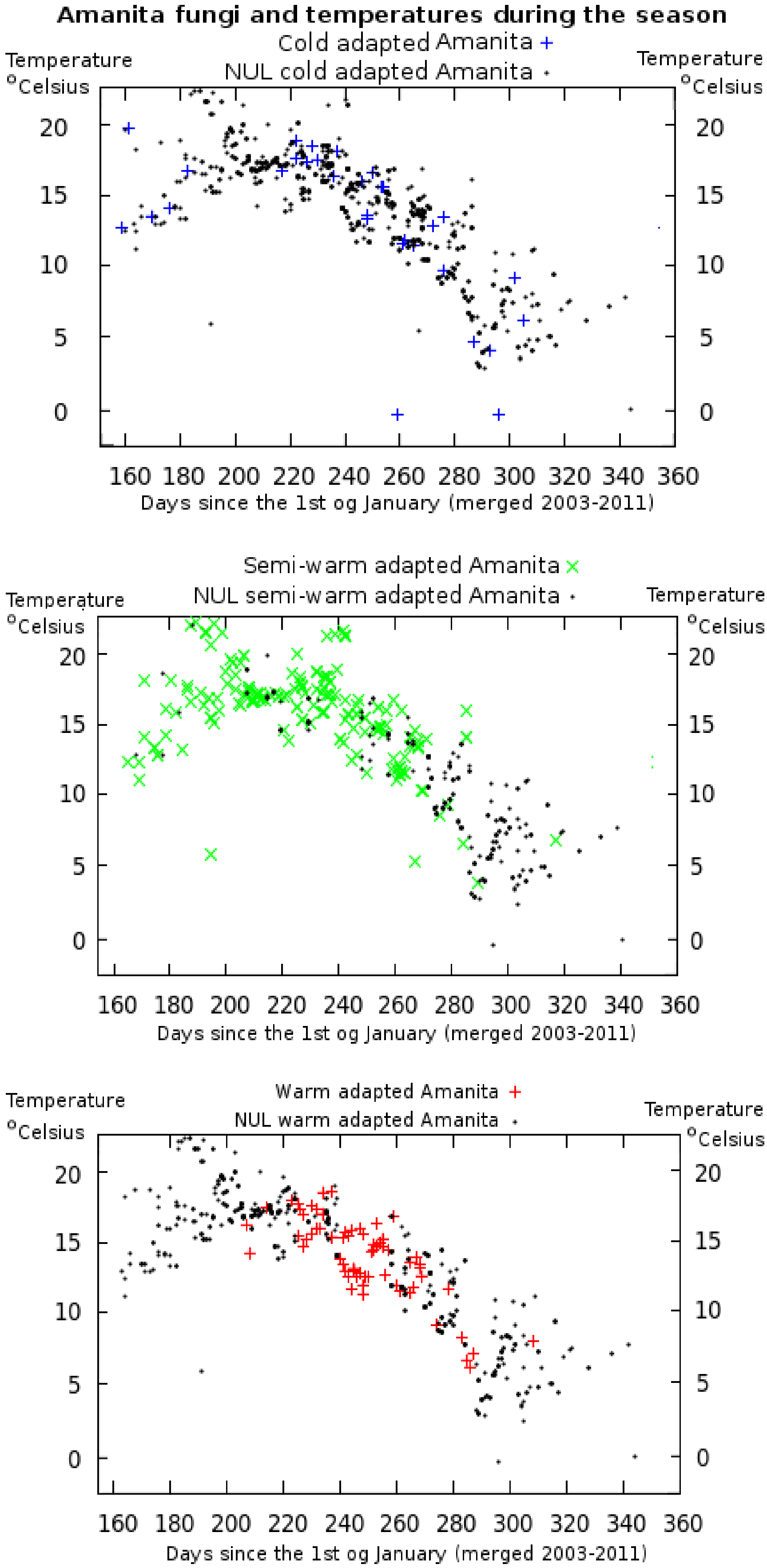


Fig.26. Comparison of temperature between the affinity of Amanita (above) and Bolete fungi (below) related to their classified climate zone(Table 1,2). The Semi-warm adapted Amanita and the warmest-adapted Bolete fungi seem to be temperature determined and indifferent to precipitation, which may future-proof their status as the most common of their genus in contrast to the Cold-adapted ones.

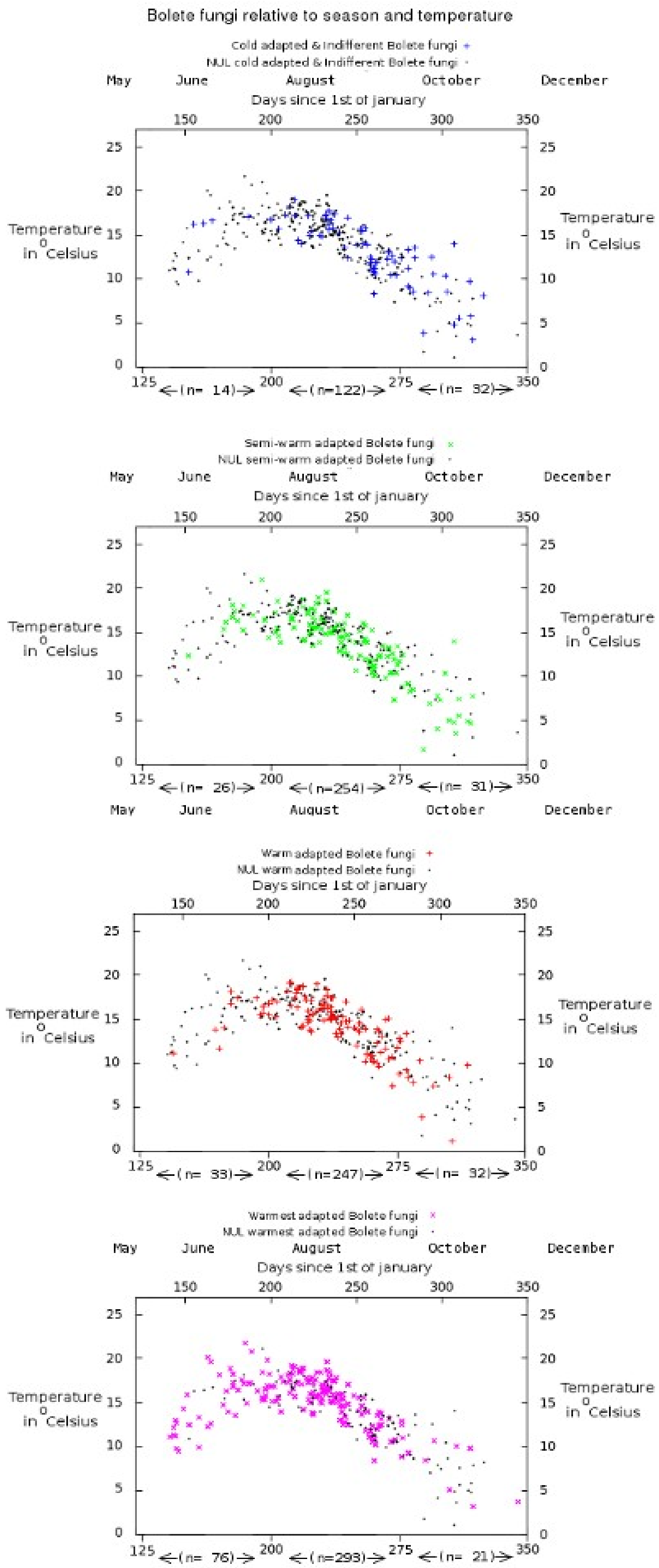


Fig.27. Bolete fungi related to their temperature according to their classified climate zone(Table 1,2).

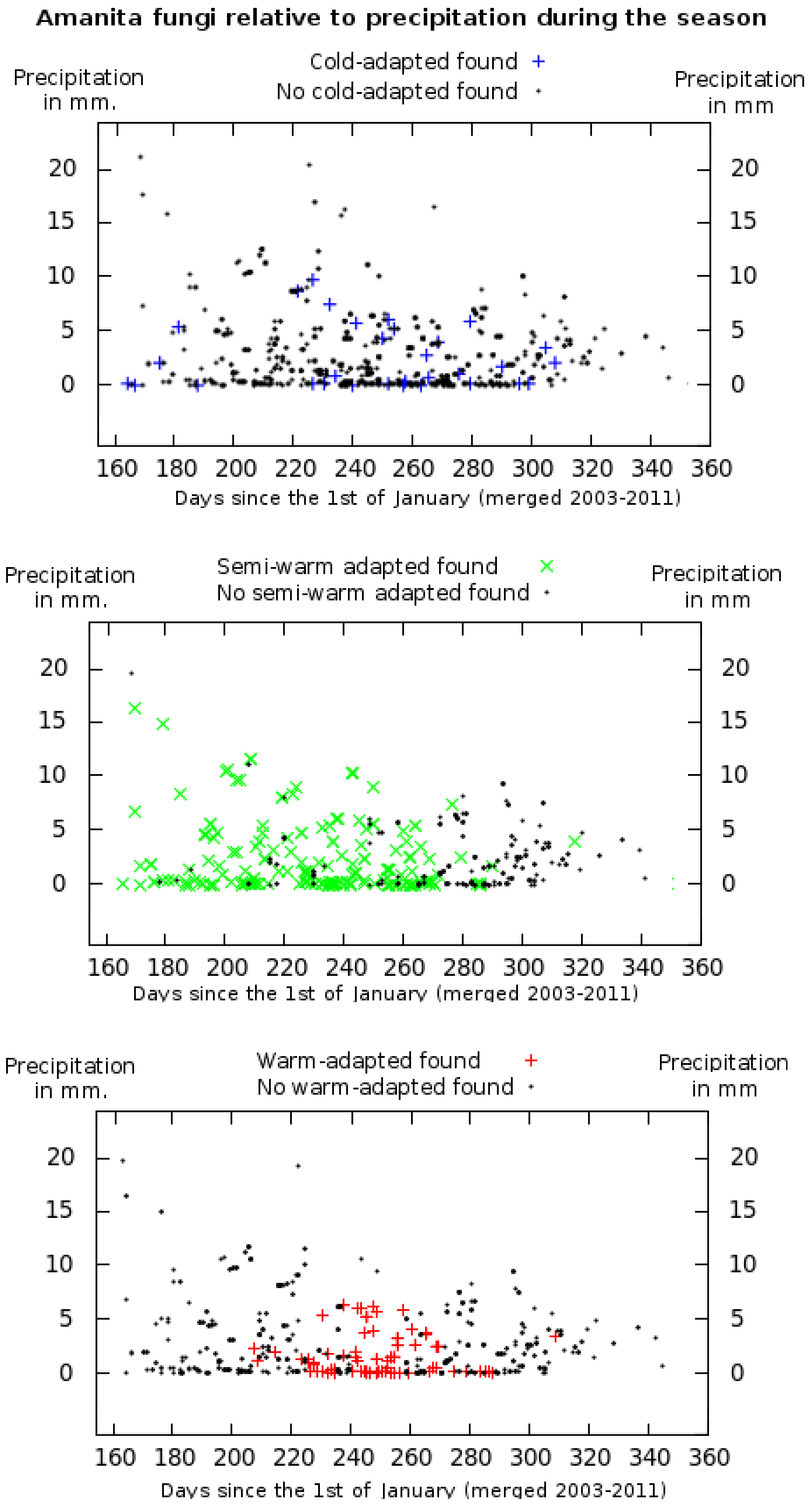


Fig.28. Comparison of precipitation between the affinity of Amanita (above) and Bolete fungi below related to their classified climate zone(Table 1,2). The Warm adapted Amanita does not seem to be precipitation determined.

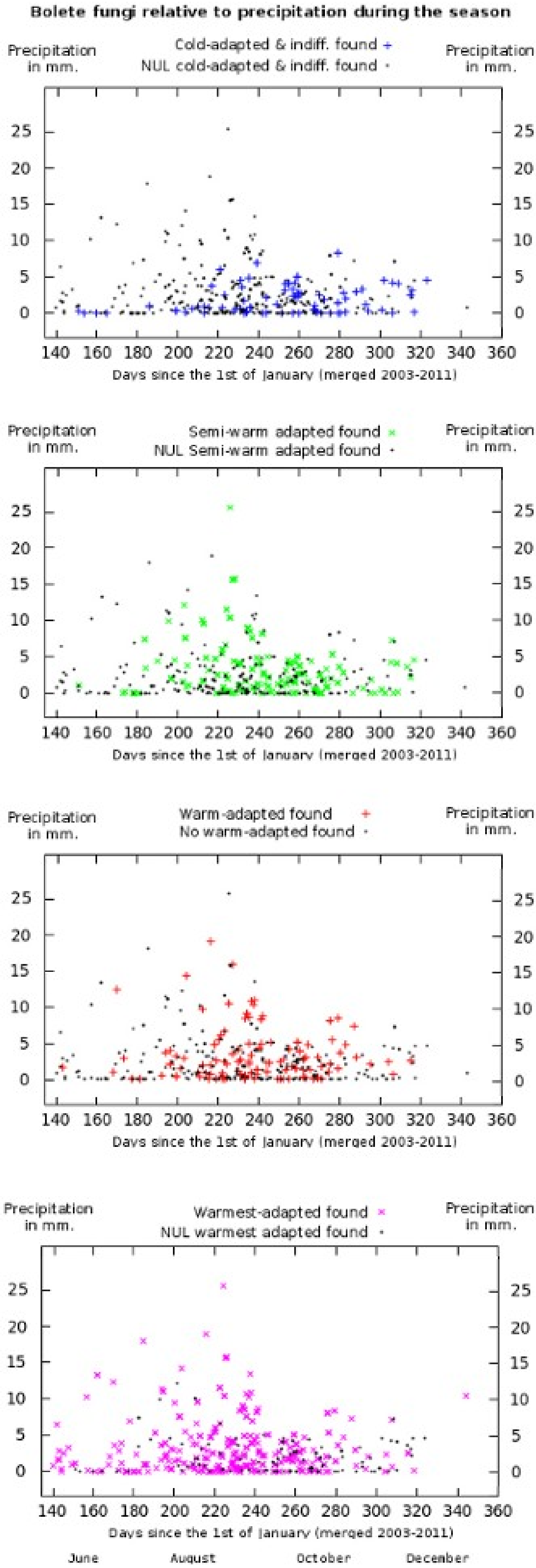


Fig.29. Bolete fungi related to their precipitation

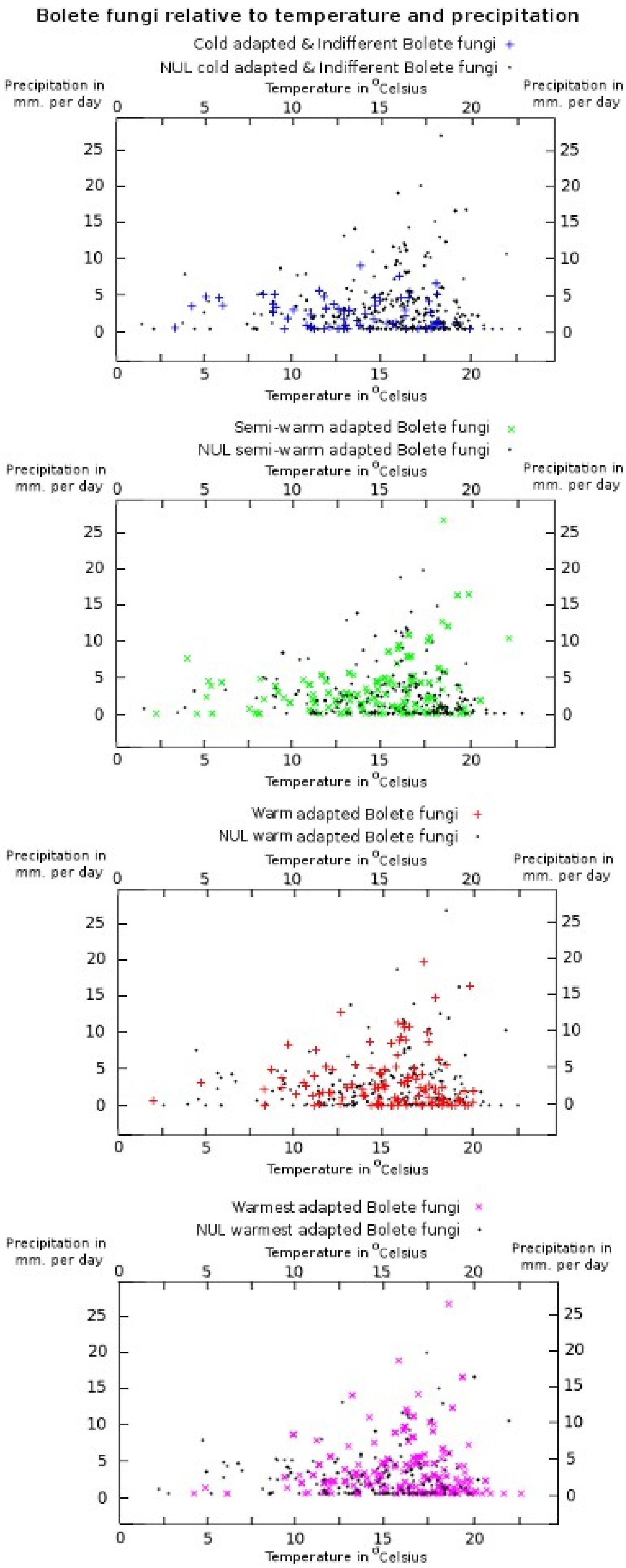


Fig.30. Bolete fungi and temperature. Cold adapted are not found at high precipitation as for the Amanita.

www.globalbioclimatics.org/form/tb_map/MN30W060.htm

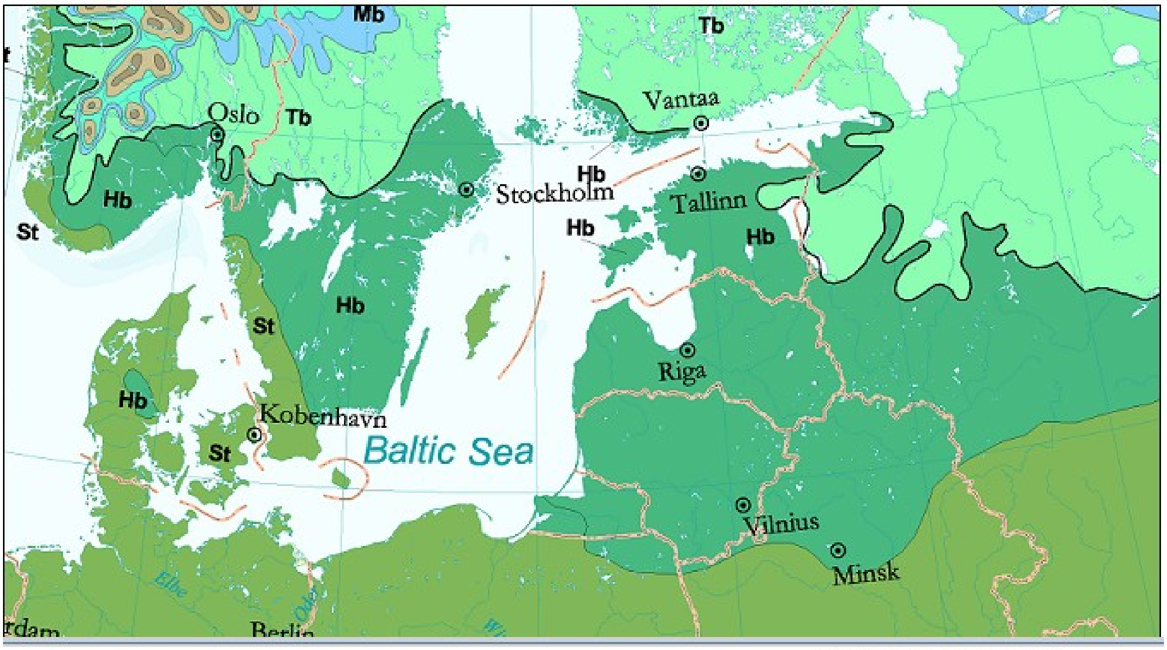
Worldwide Bioclimatic Classification System

Phytosociological Research Center

Home - CIF - Publications - Maps - Search stations - Programming - Diagnosis - Statistics

Bioclimatic Map of Europe: Thermoclimatic Belts

Go to: Bioclimates - Thermoclimates - Biogeographic Map - Legend Navigate:



		Bioclimates / Variants	Bioclimatic thresholds Itc	Tp (1)
		TEMPERATE		
It		**Infratemperate**	410 - 480	> 2350
Ism		Infra-submediterranean (2)		
Tt		**Thermotemperate**	300 - 410	> 2000
Tsm		Thermo-submediterranean (2)		
Mt		**Mesotemperate**	180 - 300	> 1400
Msm		Meso-submediterranean (2)		
St		**Supratemperate**	< 180	> 800
Ssm		Supra-submediterranean (2)		
Ot		**Orotemperate**	-	380 - 800
Osm		Oro-submediterranean (2)		
Ct		**Cryorotemperate**	-	1 - 380
Csm		**Hemiboreal** (3)		
Hb		Cryoro-submediterranean (2)	-	-

Fig.31. From local indication to mapping: Denmark has only one small hemiboreal zone near Viborg and elsewhere it is temperate. A possible loss-process of many Scandinavian cold bioclimatic zones maybe a reality, if no landscape connectivity (green zones) and climate-refuges are conserved for per-change survival of sensitive species. A climate targeted policy is urgent, but that needs migration mapping that mirror the climate change scenario of the species.

Variation in Alternaria dispersion through 27 years

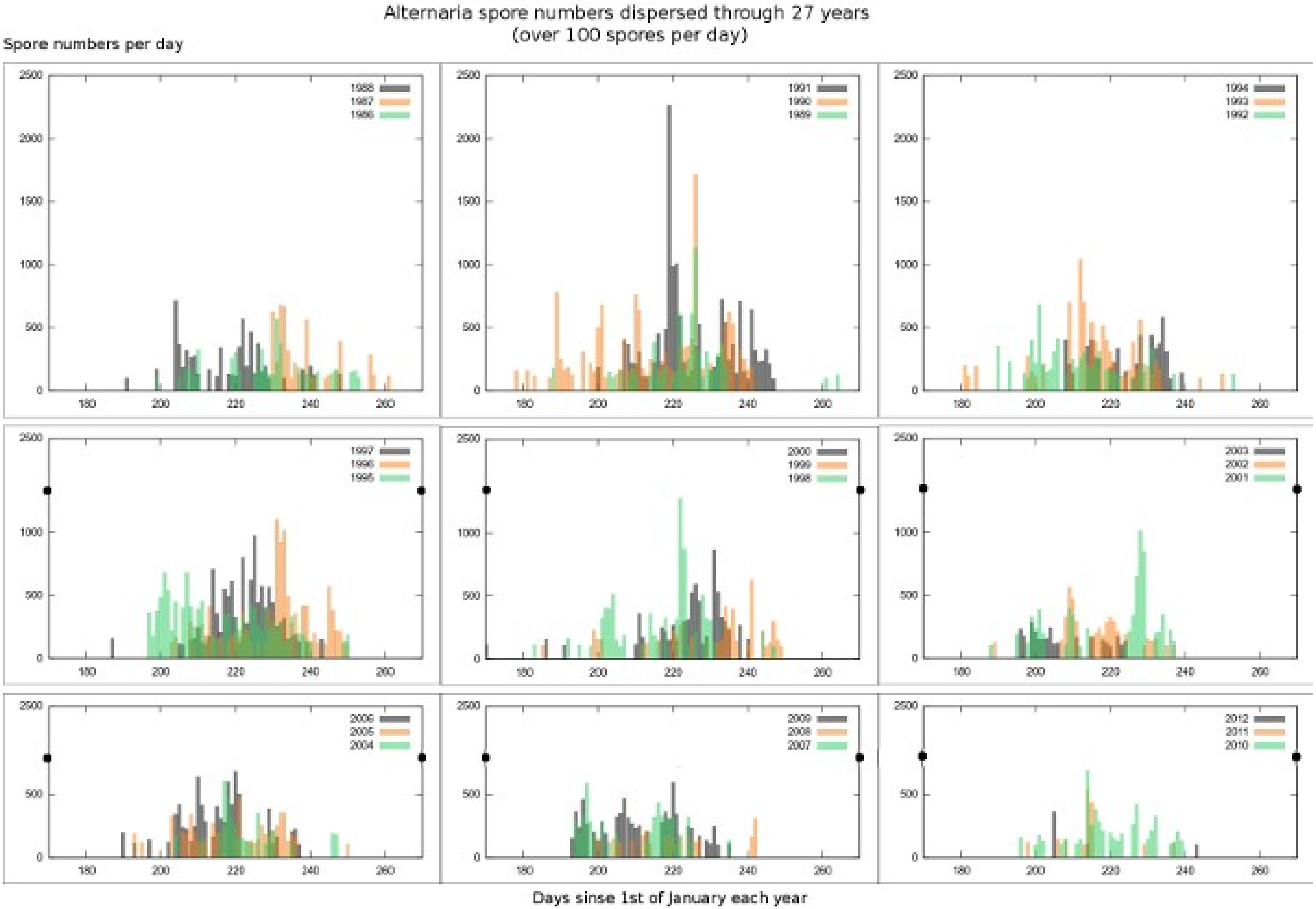


Fig. 32.

Perspectives and further to this study

Improving the indications from more and better biologic- and climatic-data

Multiple and well-defined climate parameters: Other climate parameters than the studied ones may be decisive. In principle, climate changes of local wind direction may be relevant for the wind-borne fungi. Sometimes, land-use may be more significant to organisms than climate changes, and the DMI scientists are aware of human footprints in their new climate scenario (Shuting Yong 2012). Besides, it needs further investigation to verify if the present-day mean precipitation, the relative water-content or a waterlogging value that are the most decisive water-parameter. Finally, the correlation used for the calibration could be improved, so an anova corelation routine is programmed. This corelation could be between GPS-defined & chronologically defined biologic detail-data AND modern fine gridded radar / satellite weather-data that are adjusted to older permanent weather station data.

Many indicators into a database: For the indication to be reliable, multiple indicators need to be recorded in a database. Their spatio-temporal distribution need to be analyzed statistically. Each calibrated bio-proxy should indicate local temperatures of the same and different climate zones and be tested for how well-fit they indicate recorded weather-data through a medio term period. To do so, repeated calibration in a number of habitats would substantiate our indicators, before re-finds in conserved sediments finally verify to indicate temperatures as the "above 11°C for Alternaria at peak-level" back into pre-industrial time.

The choise of bio-proxies and indication method: The more species with narrow but deviating tolerance ranges, the better the climate indications will be delimited. Besides to indicate temperatures below 0°C, living fungi like the Cantharellaceae have a high dry matter content and may so be suitable. In the other end to indicate high minimum temperatures, deviating poichilotherm organisms like lizards (Lacerta vivipara) would be a possible indicator choice.

The location of the indicators: To increase the correlation between climate tolerances and the organisms, a migration study would uncover when and where the indicating spores comes from, its route in the air and where they are settling. For that local registrations at ground level of the spores will be most helpful. According to De-Wei Li (2005), Amanita spores are found at ground level to 2.7m. At least at 3m and above different Alternaria species are frequent (Poonam Sharma, 2004) and at 15m's altitude Alternaria alternata and Cladosporium herbarum at DMI are almost alone in the counted samples. "Spores of different species exhibit characteristic circadian periodicities in their occurrence in the air spora because their method of liberation is correlated with time of day" (Kirk et al. 2008). That is a full 'dispersion and migration study' and includes the other species that disperse at ground level e.g. Alternaria solani, Amanita and the Bolete fungi and their spores.

Basic science perspectives and bioclimatic mapping

Parallel climate models build on bio-proxies may be developed or a reverse estimation of CO_2 equivalent data from the indicated temperatures can be used for verification. It provides another point of view and may render possible if the proxies or the climate model should need adjustments. As soon as parallel climate models are created, a comparison study based on bio-proxies is possible as for data-model comparison (Brewer, S. et al. 2007). That is to compare in situ climate indications achieving a resolution within hours (or days) in areas that have not been or will not tangible be registered instrumentally.

Besides, as the results of indications using bio-proxies are not using border estimates between regional and global climate modelling, any sudden jump in indicated temperatures found using bioclimatic mapping* will expose possible needs for adjustments.

While a basic physical perspective can be "how dispersion & migration of poikilotherm organism indicate the climate", the biologic counterpart will be "how dispersion & migration of poikilotherm organisms are affected by the climate". Fungi appear sufficient sensitive to the climate, and the procedure to discover such climate related movements can be the Bioclimatic mapping method*.

Applied science perspectives and bioclimatic mapping

The choice of key-organisms from sectors and bioclimatic mapping may lead to permanent exchange of climate results with the sectors.

Healthcare and welfare – Warning of allergenic stress-levels

The interrelated days where all Alternaria and climate data are registered in the 27 years period(table B) has already more days above 15°C and more days with precipitation. Increasing number of days with precipitation and high temperature may mirror why, the peak 'season' of this Nordic study deviates considerably from the Turkish study of Celenk, S. et al. (2007).
The peak-level "season" is not found outside day 180-260 and for probability indication 90% are between day 200-245. There is, however, no biologic evidents for a season (Shripad N. 2009) - due to succession or reproductively demands of microfungi. If any season, it should be co-evolution to the grasses (and cereal harvest), which, as reasoned, our data does not support. It appear more likely that the peaks arrive, when the climate conditions are suitable. Suitable microclimate conditions (although for the Bolete fungi) are stated to be "particularly humidity, soil moisture, and soil temperature" (Theirs, et al. 1994). The peak-level found here, happens consequently at temperatures above 11°C (90% above 15°C). In 15m's altitude the peak-level is found, when the precipitation is below 32mm (the 90% below 10mm). When the climate data of each hour is coincided with the peak-levels, the local correlation may be considerably improved. In stead of "it is the season, where you risk allergy", functional data-correlations with climate will help forecasting the peak-levels. It is not yet verified, however, whether the changes through the years at peak-level match changes in allergenic reactions needs verification with data of the local health inspector.
Allergy management could imply: Fungi allergists may avoid some of the pathogenic levels by living in protected flats *at high altitudes*, air out *when the precipitation starts* and *filter dry incoming air* when the fungi reach a high peak-level or escaping to 15 m's elevated buildings or cold mountain areas. Especially regarding kids, the dispersion study at ground level would be valuable (Ai-ping Zeng, 2011). The increased mold allergy found south of Denmark (Richter, M. 2014) would be less alarming, when you can avoid outbreaks, as climatic dependence of the allergens may be predicted according to weather parameters and mapped locally.

Self-aid could be provided by producing an app or expand e.g. the Naaf.no's app to predict detailed information of where and when peak-levels of allergenic fungi are feasible.
Allergy warning managers can provide very local advises, how to prevent allergic persons from being exposed to high-peak levels of spores, as they have local and updated overlay GIS-maps gathered within the Bioclimatic Maps.

Agriculture and Forestry

The *Cladosporium herbarum* & *Alternaria alternata* are captured using the Burkard volumetric spore traps at 15mś altitude. However some Alternaria especially *Alternaria solani* is a problem in agriculture, although different spore-shape and sizes may bring the spores to disperse differently. Common issues of dispersion exist and studies of Fusarium and aphids are urgent. The agriculture scientists of Foulum take an interest in registering the airborne spores of each species at ground level. Alternaria may disperse kilometers, while some Amanita "less than 5% of basidiospores released were dispersed to the second location 5.2 m away and 2.7 m above the basidiomata" (De-Wei Li 2005). "For many fungi, horizontal spore concentration in air is normally minimal at 100-200 m from the source and the vertical concentration decreases logarithmically with height above ground. Fungal spore viability is important in determining migration capacity: rusts spores remain viable for many days and can carry infections great distances" (Kirk et al. 2008).
A study of the airborne pollen & fungi related to coming methodological incidences - now and during climate changes - may increase basic climate science plus prevent crop reduction in the years to come.
Agriculture and Forest consultants have options to become timely aware of the possibilities of

changing crop- and tree-species according to weather- and climate-changes using the Bioclimatic Mapping facility. Changes in mycorrhiza and in pests becomes more visible when the consultants become able to follow those over time (Bellgard, S.E. & Williams, S. E. 2011) visualized in the Bioclimatic Maps.

Land-use & Conservation: Mapping where organisms may migrate & adapt

Agronomist and other planners become able to locate and predict more extreme weather situations from Bioclimatic Maps, as it show sudden migration of the bio-indicators. The managers will become more capable to economize by the subsidizing example of the deciduous replanting since the tree-fall in Denmark.

When a number of bioclimatic-indicators are the same for different area, it indicates similar conditions for the organisms. It could be worthwhile to study if deviating landscapes belong to the same biogeographic category (a known example is tropic mountains and subarctic zones).

As adaptation to tolerable conditions seem reflected in the areas' bio-indicators, refinds of these indicators in different locations may uncover very local climate variations. That way new landscape connectivity areas may be discovered for the species to adapt. A pattern of matching climatic areas to endangered species will hopefully be found as a *sequence of suitable and coherent locations for adaptations.* Information of the red-listed species may be added to Bioclimatic Maps* to gain a survey of landscape connectivity areas and refugia for conservation and land-use planning. This may dramatically change priorities of which areas that should be conserved and which should remain for agriculture.

Landscape and Conservation authorities will benefit from the mapping, when they can document that a specific landscape connectivity pathway is a passage for multiple sensitive species in their migration to reach a climate-refuge - that could be a sanctuary, where they perchance may adapt. Yet a huge issue to be prioritized (Rudnick, D.A. 2012).

Possible pre-historical outcome may teach us about our future: When precisely around year 1000 and where did the "Medieval Warm Period" break the temperature-increase into a "Little Ice Age (IPCC 2001)? How did the flora and fauna look like and adapt? The temperature in industrial period may increase 10-100 times as fast as the warming after the ice-age, even though short periods of natural cooling may beat back some of the expected mass-extinction of species. Mapping of different microclimate areas due to decisive climate parameters may prevent demotivating surprises such as 30% less birds found the last 20 years. Bioclimatic mapping may show this step by step, plus expose new crops and strategies for land-use. Better predictions will help *food-chain stability* and *land use management* such as organic farming as elements of green corridors to climate-refuges for climate sensitive species gaining time for some species to adapt.

Public nature-awareness & applied Bioclimatic mapping perspectives.

The Bioclimatic mapping facility will be a very practicable self-aid facility for the public by use of a smartphone, as well as for members of the above sectors: Imagining the Bioclimatic Mapping facility to be funktional in an area, any person with a smart-phone can search for e.g. eatable fungi. Depending of his/her GPS-location, a map will appear, showing the probability of the eatable fungi and the potential risks of confusion for the actual time and place. The database behind the map utilize finds from previous years - delivered and reviewed the open-source way by non-professionals, too (like svampeatlas.dk shown like the kameraspotter app animated as the cloud radar videos). Besides, the map will be based on: Bio-proxies indicating the local micro-climate, weather conditions for the last 10 days, 'seasonal' changes, local shadow, wind-directions, soil type, vegetation, time of year, etc. Many of these parameters are already put into system (such as the soil types and the Fine-Scale Road Stretch Forecasting).

On a smartphone, another person may write 'allergy', and the local and seasonal risks of e.g. *Alternaria alternata* appear on the screen map. A gardener may writes 'potato' to possible change to a late crop of potatoes. The outcome could be the probability of success for this particular location and timing due to risk factors of the molds eg. Alternaria solani. Dependence on soil type, former crops, vicinity to neighbour plants, weather etc. are taken into account, as it is included in the local

GPS-information behind the Bioclimatic Maps. Bioclimatic Mapping is used for awareness by UNIBAS (Geeson, N. 2004).

Adding more indicator-species to the database and maps behind the Bioclimatic Mapping facility is straight forward and is scalable to suit different stakeholders' needs and their budgets. Collaborators are found, climate-, weather-models and data are already available for 23 species of birds, 6 species of insects, 22 muchorrizal fungi, and pollen- & spore-registrations from 2 stations at DMI.dk and 12 at NAAF.no along with nearby weatherstation-data or reconstructed weather- and climate-data...