Faculteit Bouwkunde
Vakgroep
Fysische aspecten van
de gebouwde omgeving

Technische
Universiteit
Eindhoven

SAMENVATTING SCHIMMELONDERZOEK

a.m.s. weersink
ir. p.c.g. adan

rapport : 88.04.K
datum : 880324

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Uitvoerder:

Technische Universiteit Eindhoven
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A.M.S.Weersink
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Dit rapport bevat twee samenvattingen van resultaten van onderzoek naar schimmelontwikkeling op bouwconstructies. Beide onderzoeken zijn verricht in het kader van het onderzoek 'De vochthuishouding van gebouwen', dat binnen de Voorwaardelijke Financiering van de vakgroep FAGO wordt uitgevoerd.
In het kader van informatieoverdracht in de IEA-Annex XIV 'Condensation and Energy', worden de onderzoeken in de bijeenkomst te Glasgow van 11 tot 13 april 1988 gepresenteerd. De samenvattingen vervullen daar dan een voorbeeldfunctie om de overdracht van informatie tussen de in de Annex XIV participerende landen te structureren en te stimuleren. Deze informatieoverdracht is essentieel voor het slagen van het onderzoekplan.
Deze samenvattingen zijn vervaardigd in opdracht van de Stichting Projektbeheerbureau Energieonderzoek (PEO).

PROJECTDESCRIPTION

1. Title
Sampling and Analysis of Mould Growth in Buildings;
Preliminary Study in Three Dwellings

2. Research by
Eindhoven University of Technology (TUE). Faculty of Building Science. Trade Group FAGO (Physical Aspects of the Built Environment).
Ir.O.C.G. Adan, A.M.S.Weersink

3. Requested by
Eindhoven University of Technology (TUE). Trade Group FAGO.

4. Availibility Report
Following reports are available:
-'Bemonstering en analyse van schimmelaantasting van bouwconstructies; voorstudie in drie woningen' [in Dutch];
FAGO report 87.11.K (date 870923)
-'Sampling and analysis of mould growth in buildings; preliminary study in three dwellings'. CIB.W40 Meeting, August 31 th- September 2nd 1987 [in English]

5. In cooperation with
-**Rijksgebouwendienst** (Government Buildings Department)
Afdeling bouwfysica (Section Building Physics)
-**RUU** (Utrecht State University)- Laboratory for Ectoparasitology and Dwelling Hygiene.

6. Connection with other projects
The project forms part of the research entitled 'The dampness Economy of Buildings' (TUE; researcher: ir.O.C.G.Adan).

7. Purpose/Commend of the research
An important part of the research 'The Dampness Economy of Buildings' consists of investigations into the causes of visible mould growth on building materials and furniture.
To handle mildew corrosion (cure and prevention) knowledge of both biological and physical processes is necessary. Adequate diagnostic techniques therefore are required.

8. Aim of the project/Target group
1. To become conversant with terminologies, measuring and analysing methods in Mycology and Building Physics by both building physicists and biologists, in order to improve future communication.
2. Formulation of a joint (biological-building technical) research-project. Diagnostic techniques to get biologic background information must be developed. The present organisms and the pattern of the damage may inform about the microclimate on the spot of the problem, maybe from past and present.

9. Time-schedule
Initiation date: March 1987
End : April 1987
Status : Concluded

10. Short description of the project

The project consists of four parts:

1. Introduction to mycology from a building physical point of view;
2. In three dwellings, dealing with mildew-problems, a few sampling and analytical methods that orginate in veterinary and medical diagnoses, were carried out;
3. Evaluation of the results;
4. Formulation of a proposal for continuing research.

1. Introduction to the world of moulds

Briefly discussed are:

-Germination, growth and reproduction of moulds in general;
-a classification of fungi between other organisms;
-taxonomy and nomenclature;
-determination of moulds;
-building up and composition of a cell;
-environmental factors influencing the development of fungi in general, metabolism (assimilation/dissimilation), adaptation to environmental circumstances.

2. In three dwellings, dealing with mildew-problems, a few sampling and analytical methods that orginate in veterinary and medical diagnoses, were carried out

Sampling methods

1. tape-method
   Scotch Magic Tape is firmly pressed onto an 'affected' spot. Sampling tapes are stucked onto slides for transport.
2. scrapings-method
   If, for utilisation technical reasons, no material can be collected via the tape-method, mould-attacked (contaminated) surface material is scraped off.
3. removal of entomological material
   Small organisms (insects and spider-like creatures) on or in mould-contaminated patches are removed with tweezers or stucked on tape.
4. house dust sample-method
   In case of very badly mildew-affected spots, contamination of house-dust is expected. It is supposed that the concentration of damage-causing species will be remarkably high. House-dust is strongly sucked out of carpets with a vacuum cleaner.

Analytical methods

tape

One cm^2 of an infected piece of tape is pressed four times on a graft bed. M82, M95, DTM and Nickerson were used for cultivation. The duration of incubation-period (at 20 Celcius) depended on initial period and growth-rate. The number of colonies per species were counted. Key tables are used to determine the species. Of several attached patches, direct preparations were factured.

House dust samples

dilution-plate-method

4*.02 gram of one house-dust sample is suspended in 55% sucrose solution. Dependent on the quantity of sucrose solution, 1:1, 1:8, 1:64 sucrose solutions are obtained (see figure).
These solutions are strongly shaked. 1 ml is grafted out over a

bed and is incubated. Hereafter, colonies are counted and determined. The quantities of living diaspores per species in house-dust are calculated.

apizyme-test

An apizyme-test has been developed to show 19 enzymes (possibly secreted by moulds). Reagents are added to diluted house-dust. The intensity of discolouration of these reagents, is used as a semi-quantitative indication of enzymeconcentrations in a housedust sample.

guanine-test

Guanine is secreted by spider-like creatures (mites) and can be of importance for allergic reactions. The guanine-test is used to determine the amount of guanine in a weighted amount of dust-sample. A test-liquid is prepared. The guanine content of a house-dust-sample is calculated from the relationship of the extinction of light (λ=490nm) of a prepared test liquid and a reference-liquid.

urine-acid-test

Urine-acid is secreted by insects. The urine-acid content of a housedust-sample is calculated from the relationship of the extinction of light (λ =340nm) of a prepared test liquid and a reference-liquid.

Sampling carried out

Dwelling in Muiden

1. Scraping and two tapesamples of a stucco wall (south-west) corner with black discolouration of the side gable level with the ceiling with a green/yellow discolouration;
2. Animals in the stucco work in the south-west corner:2 living, 4 death;
3. Dust-sample: living room carpet, 15*0.5 m^2 suction surface approx. 2 meters from the problem corner.

Dwelling in Doorn

1. Nine tape-samples of mildew corrosion in the inside wall behind a mirror in the bedroom on this floor;
2. Vacuum cleaner samples: bedroom carpet ground floor approx. 0.5*1.0m^2 suction surface.

Dwelling in Wageningen

1. Twelve tape samples of mildew corrosion in the three-dimensional corner of the front gable, side gable and floor in the living room.

11. Results/ Current situation

Results of the analyses are supplemented in figures.

Evaluation of methods used

tape method

-Collection of diaspores is difficult when woolly structures are absent or not within easy reach;
-assessment of direct preparations only makes determination of genus possible. To determine the species, cultivation on standard media is still necessary;
-sampling must take place at representative locations.Different climate conditions seem to stimulate growth of different speciess. In this study sampling was carried out at random locations. How far these locations have been representative is unknown;
-problems occuring during the analysis:

.high concentrations of grafted diaspores and high growth-rate hindered counting of colonies and determination;
.beds differ strongly from environmental conditions which gave rise to the problem. Determination of 'damage-causing'-species is difficult; contaminants are often disturbing.

scrapings-method

-problems which occured during the analysis:
.high concentrations of diaspores and rapid growth hinders the counting of colonies and determination;
.it's been impossible to distinguish between damage-causing species and contaminants in culture.

entomological material

-On the basis of the colonies developed in culture, it couldn't be established that these have been the damage-causing moulds.

House-dust-methods

dilution-plate-method

-The assumption that the damage-causing fungus-species cause the highest concentration living diaspores in housedust, has not been confirmed.

apizyme

-A relationship exists between the avoidance of certain enzymes and the fungusspecies. Qualitative and quantitative assessment of a sample could be possible.

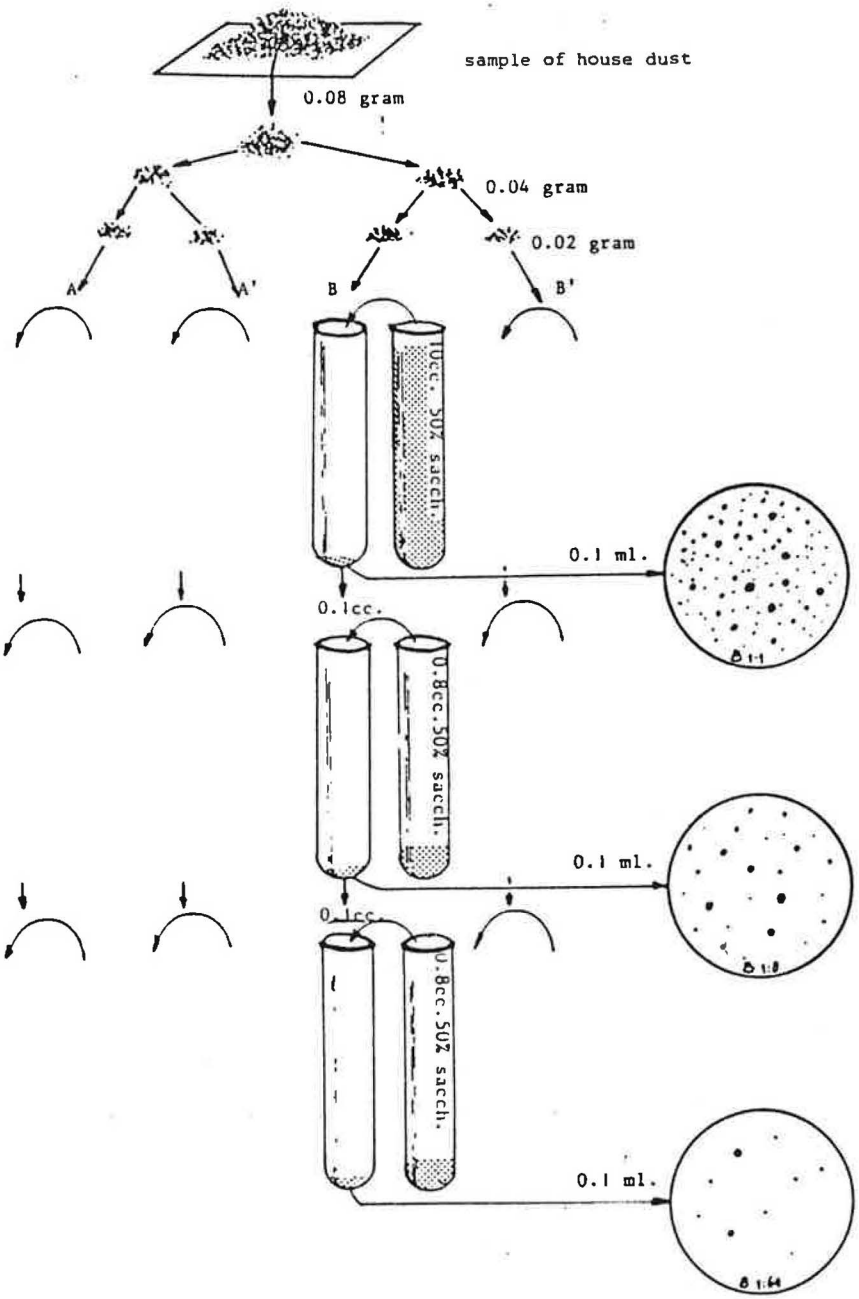
urine-acid-test

-The test won't contribute to the diagnosis of the mouldproblem.

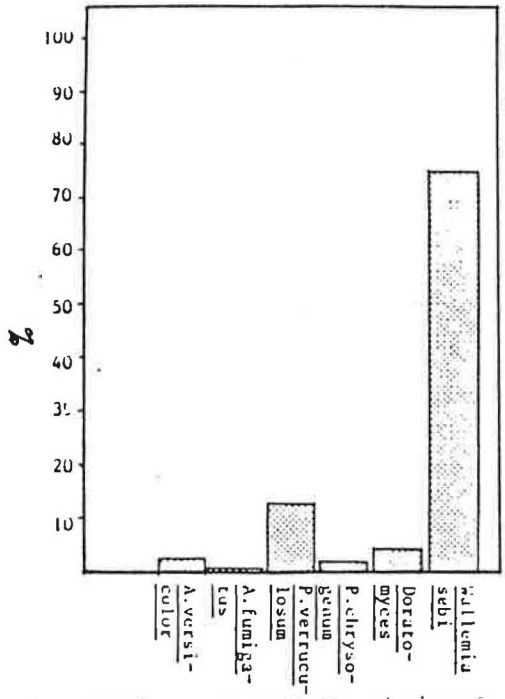
guanine-test

-Although no contribution to the diagnosis of visible mouldgrowth on surfaces of building materials will be obtained, the guanine-concentration may give any indication of the general 'humidity condition' in the dwelling.

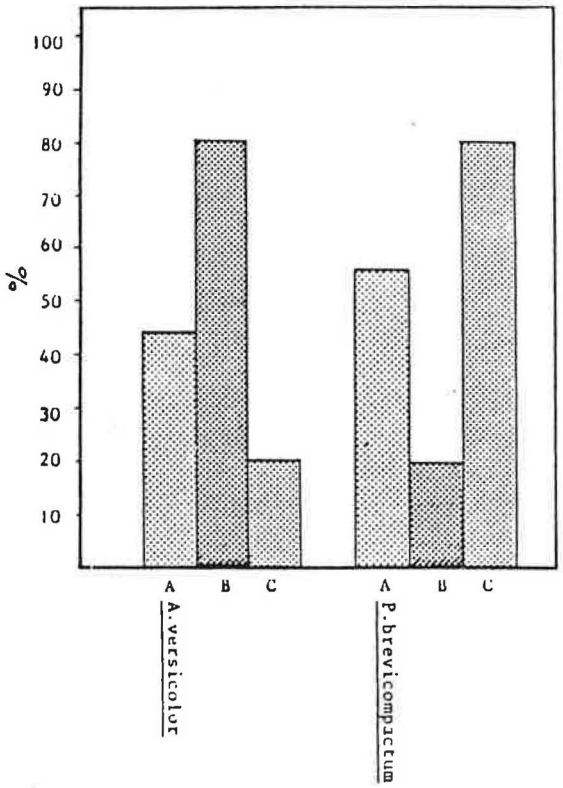
The report contains a proposal for continuing research.



Dilution plate method.



Scrapings-method MUIDEN: colonies of determined species (14 days cultivation on Malt 95)



Tape-method DOORN: colonies of determined species (3 days cultivation on Malt 95)

The amount of guanine in 2 samples of housedust (nmol/gram housedust)

Serie	Muiden	Doorn
A	5	9
B	6	6

The amount of urine acid in 2 samples of housedust (mg/dl).

Serie	Muiden	Doorn
A	0.2	0
B	0	0

Apizyme-test MUIDEN

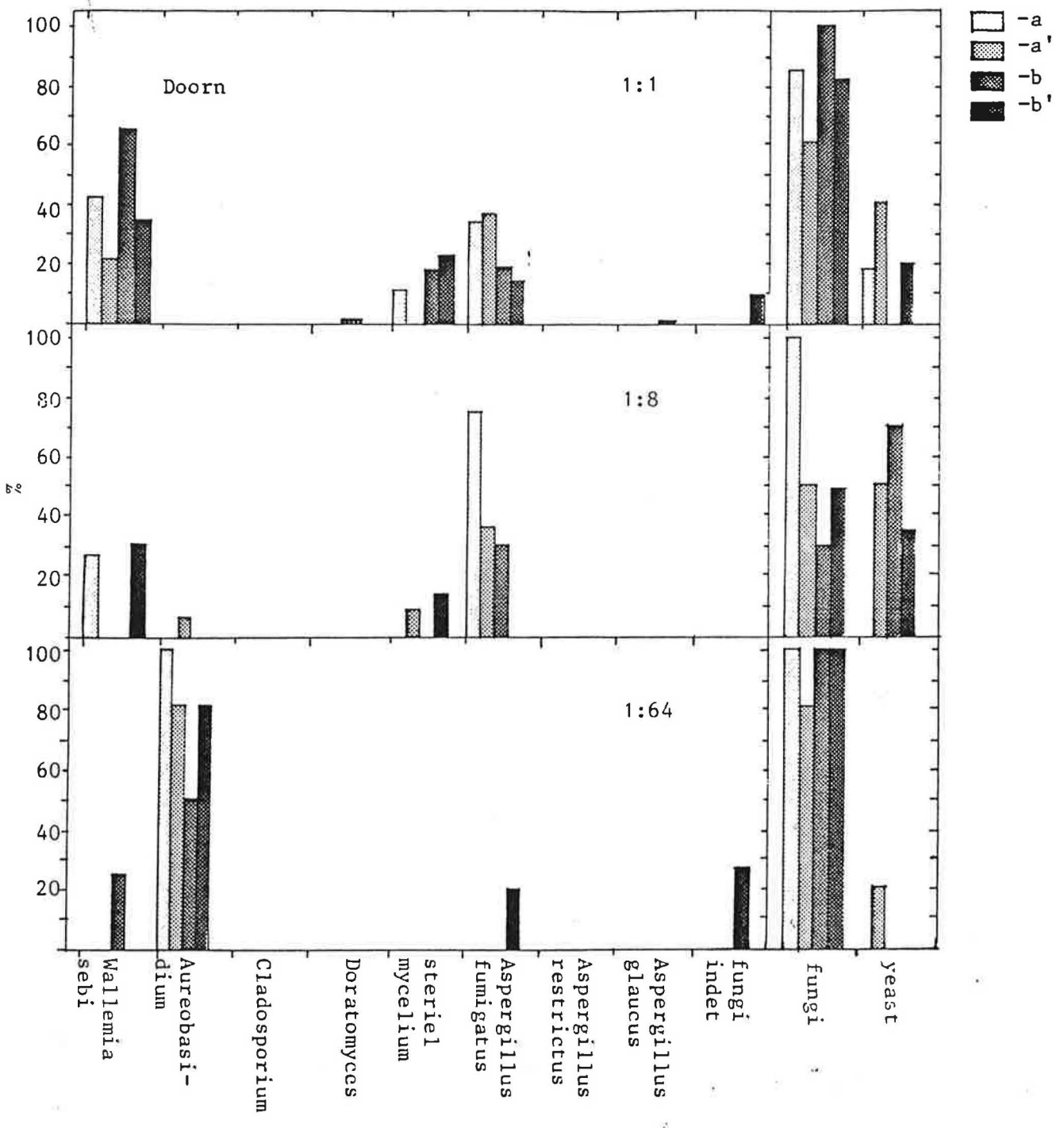
-----> enzyme	alkalische fosfatase	esterase (C4)	lipase esterase (C8)	lipase (C14)	leucine arylamidase	valine arylamidase	cystine arylamidase	trypsine	chymotrypsine	zure fosfatase	Ndaphtol-AS-BI-fosfo-hydrolase	alfa-galactosidase	beta-galactosidase	beta-blycuronidase	alfa-glucosidase	beta-glucosidase	N-acetyl-beta-glucosa-midase	alfa-mannosidase	alfa fucosidase
-----> discolouration																			
MA	2	1	1	0	.5	0	0	0	0	2	2	0	.5	0	0	1	1	0	0
MB	2	1	1	0	.5	0	0	0	0	3	2	0	0	0	0	1	.5	0	0

MA, MB = series

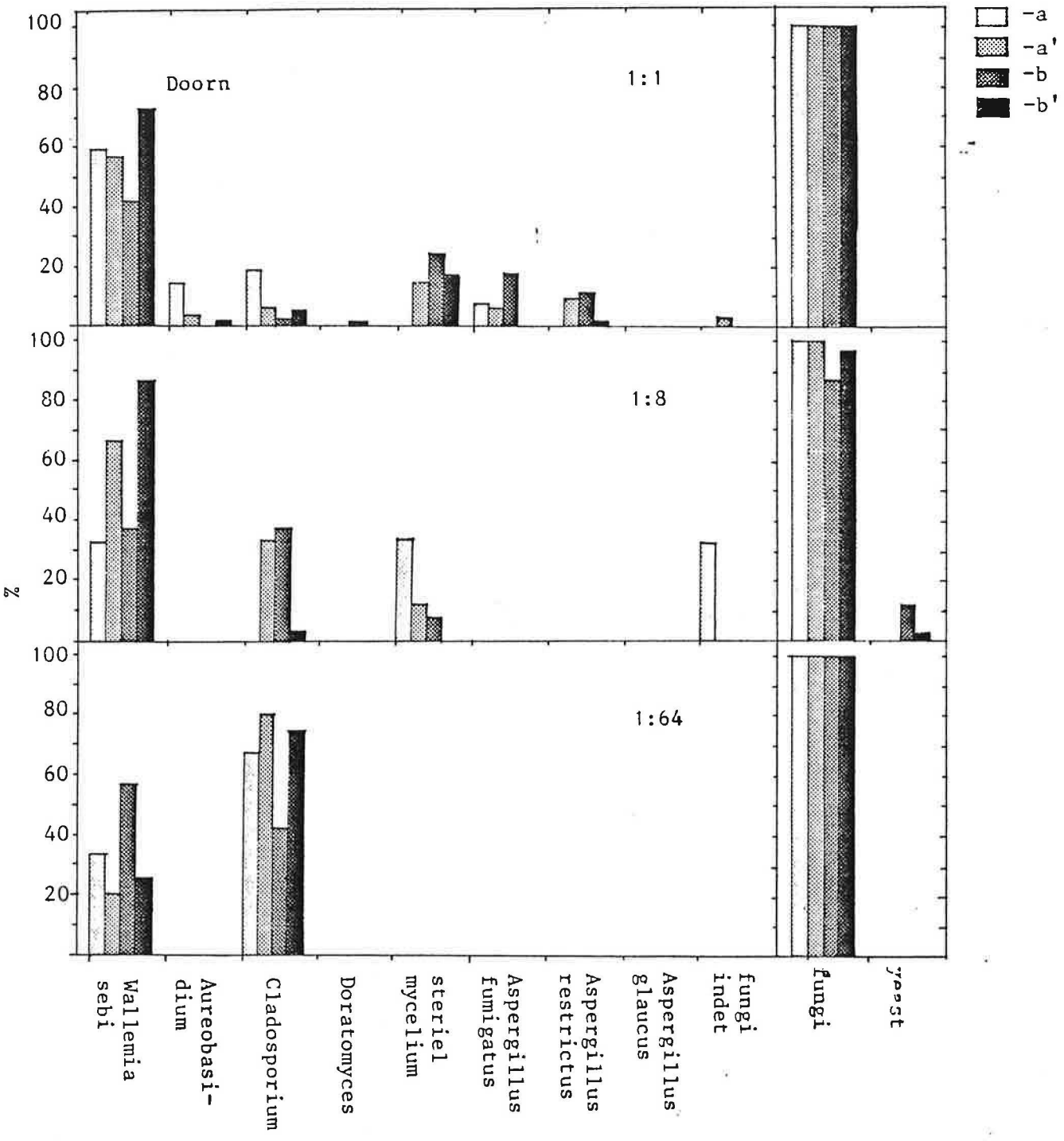
Apizyme-test DOORN

-----> enzyme	alkalische fosfatase	esterase (C4)	lipase esterase (C8)	lipase (C14)	leucine arylamidase	valine arylamidase	cystine arylamidase	trypsine	chymotrypsine	zure fosfatase	Ndaphtol-AS-BI-fosfo-hydrolase	alfa-galactosidase	beta-galactosidase	beta-blycuronidase	alfa-glucosidase	beta-glucosidase	N-acetyl-beta-glucosa-midase	alfa-mannosidase	alfa-fucosidase
-----> discolouration																			
DA	2	1	1	0	1	0	0	0	0	4	3	0	1	.5	0	1	2	0	0
DB	3	1	1	0	2	0	0	0	0	5	3	0	1	.5	0	1	1	0	0

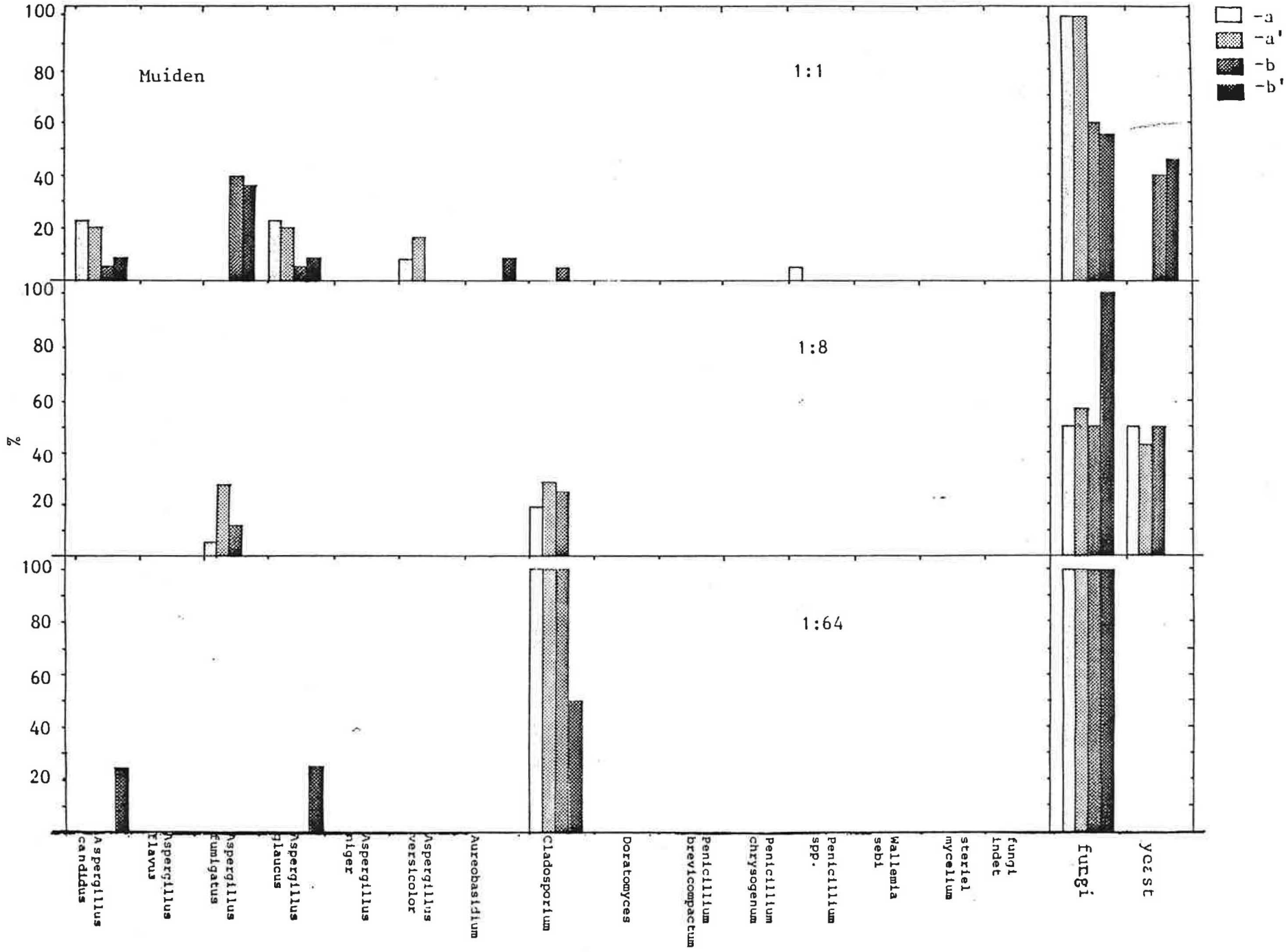
DA, DB = series



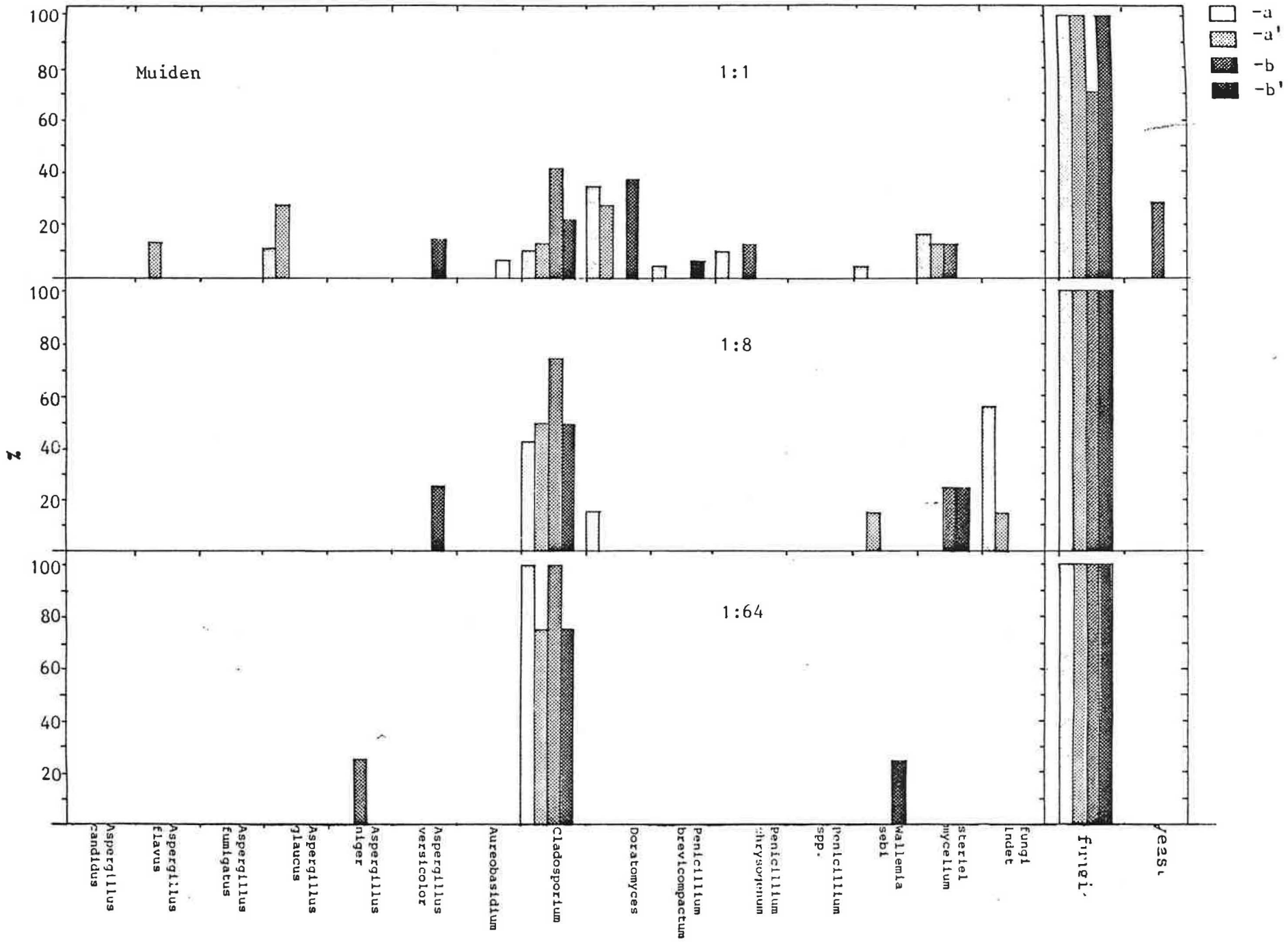
Colonies of determined species in housedust 'Doorn'
(14-days cultivation on Malt 82; dilutions a:1,1:8,1:64,
series a,a',b,b')



Colonies of determined species in housedust 'Doorn'
(14-days cultivation on Malt 95; dilutions 1:1,1:8,1:64,
series a,a',b,b')



f. Colonies of determined species in housedust 'Muiden' (13-days cultivation on Malt 82; dilutions 1:1, 1:8, 1:64, series a,a',b,b')



f Colonies of determined species in housedust 'Muiden' (13-days cultivation on Malt 95; dilutions 1:1,1:8,1:64, series a,a',b,b')

PROJECTDESCRIPTION

1. Title
Environmental factors influencing germination,growth and sporulation of Fungi; a literature survey.

2. Research by
Eindhoven University of Technology (TUE). Faculty of Building Science. Trade Group FAGO (Physical Aspects of the Built Environment).
A.M.S.Weersink

3. Requested by
Eindhoven University of Technology (TUE), Trade Group FAGO.

4. Availibility report
Following report is available:
'Invloeden van milieucondities op ontkieming, groei en sporulatie van schimmels; resultaten van een literatuurstudie' [in Dutch]
FAGO report 87.20.K [date 871116]

5. In cooperation with
RUU (Utrecht State University)
IUW i.o. 'Woning en Gezondheid' (Inter University Working Group 'House and Health');Cooperation between Trade Group FAGO (TUE) and Trade Group Dermatology -Laboratory for Ectoparasitology and Dwelling Hygiene (RUU).

6. Connection with other projects
The project forms part of the research entitled 'The dampness Economy of Buildings' (TUE, researcher: ir.O.C.G.Adan)

7. Purpose/Commend of the Research
In behalf of the partial study of the causes of visible mouldgrowth of building (surface)materials and furniture, knowledge of influential environmental factors on development of damage-causing fungi is necessary.

8. Aim of the project/ Target Group
Aims of the project are:
1. Getting some insight in effects of (combinated) environmental conditions on fungal development in general;
2. Collection of some numerical data concerning environmental factors influencing germination, growth and sporulation of fungi;
3. Determining the usability of the collected data for cure and prevention of mouldgrowth on constructional materials and furniture.

9. Time schedule
Initiation date: 20. July 1987
End date: 7. August 1987
Status : Concluded

10. Short description of the project
In libraries of the Utrecht State University, Amsterdam University and Eindhoven University of Technology, (micro) biological reviews and books dealing with ecology and physiology of fungi have been collected. Factors influencing germination, growth and

sporulation of fungi have been gathered. More specific, numerical data with respect to 27 species which are expected to be damage-causing, have been collected.

11. Results/ Current situation

Influential factors in fungal-development (germination/ growth/ sporulation) are:
-water availibility (water, damp), temperature, food supply (especially organic C and N compounds), acidity of the substrate, carbondioxyd and oxygen concentrations, chemicals (toxicants) in the substrate, light/radiation, air velocity, interactions of microbes, condition of the spores/ fungus-plant.
Used literature-sources give few informations of influential factors like CO_2/ O_2, radiation, air velocity and interaction of microbes.
All factors together define the initial period, growth rate of germtubes and hyphens, and spore-release. Especially the combination of temperature and water-availibility seems to be influential on cell-activity during germination and growth. Remaining factors stimulate or inhibit germination and growth. Fungal development is not fully stunted by these factors, unless they are very unfavourable. Favourable and unfavourable conditions differ between species, even between species within the same genus.
Influential factors all together define minimum, optimum and maximum points of temperature, R.H./wateractivity, pH, CO2/O2-concentrations, nutrition and radiation doses. This is why minimum and maximum situations of one species sometimes vary a lot. The position of the optimum seems rather stable. If one (or more) factors, especially humidity and temperature, become less favourable, the growth-range is narrowing.
Comparison of minimum conditions of germination, growth and sporulation is a difficult task, because experimental conditions often aren't comparative. Our survey showed that often only minimum, optimum and maximum are represented.
In comparison with growth, the minimum conditions to initiate germination can be equal, more stringent or less stringent, depending on the species and maybe on usage of different definitions of 'germination' and 'growth' by researchers. Minimal wateractivities of different species vary from 0.63 to 0.999.
Most experiments have been carried out under stationary circumstances. For the time being, effects of dynamical conditions on germination, growth and sporulation are unknown.

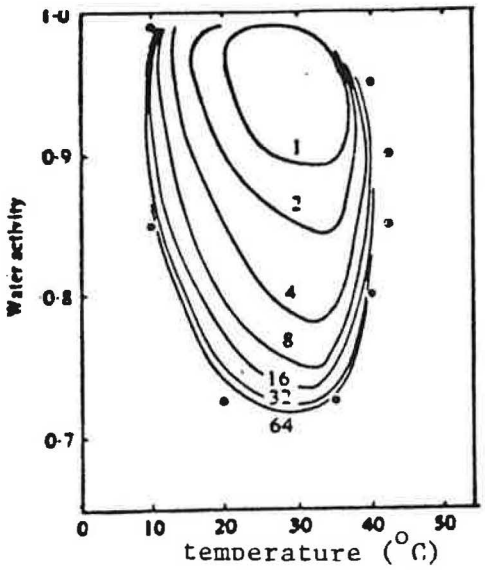
Usefulness of gathered data to Building Practice

The literature-survey resulted in only a few data with respect to moulddevelopment on surfaces of buildingmaterials. Most experimental circumstances were not comparative with those in building practice:
1. substrate: Results often present initiation-period, germination and growth-rate on standard media and foodproducts. The surface-roughness and composition of the subsoil is not comparative with building-materials. Among other things, like possible presence of toxicants and inadequate food-supply, water-absorbing and water-fenacity properties and thus water-supply, may differ strongly between experimental conditions (culture media) and conditions in building practice.
2. temperature: Experiments mostly are carried out at 'higher temperatures' ($\pm$ 25-30 Celcius), only a few within the range of 5-15 Celcius. In building-practice abundant mould growth often has been seen in last mentioned range. The optimum-range mostly

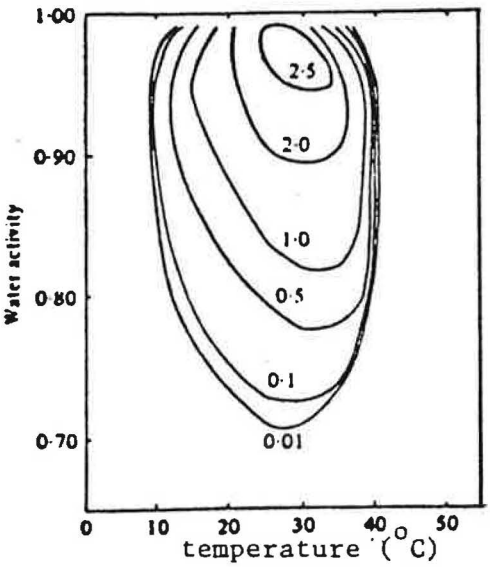
lays near 20 - 25 Celcius. Low surface-temperatures possibly imply higher minimum water-supply to stimulate spore-germination and mould growth.
3. stationary conditions: Environmental conditions in Building Practice aren't stationary. The survey showed very few results of dynamical influences on fungal-growth.

Influence of some remaining factors
4. acidity: Up till now, influences of acidity in building practice are hardly known. Combined with inadequate water-supply and unfavourable temperature, non-optimum acidity of the substrate could inhibit fungal development. Extremely high and low acidity-levels prevent germination and growth.
5. carbondioxyd/oxygen concentrations: In Building Practice excessive high or low CO_2/O_2 pressures aren't expectable, so these factors aren't expected to be stunting.
6. light and radiation: Among other things, influences of radiation intensity and spectral composition depend on the species (and precense of some pigmenttypes). Several wavelenghts could stimulate internal cell-activity as well as inhibit (biochemical) reactions. Because of the fact that fungi are heterotrophic living organisms, light isn't necessary to assimilate organic carbon compounds.
7. air velocity: Data with respect to effects of airvelocity (combined with relative air humidity/ temperature) on fungal development haven't been found.
8. interactions of microbes: No data have been found.
9. adaptation: Unknown is in what way adaptation processes play a role in fungal-development in building practice.

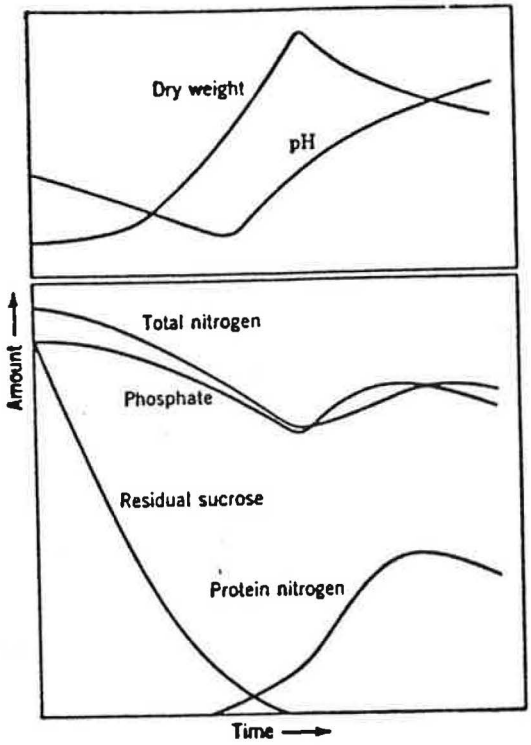
In studying mould-problems in buildings, present numerical data (of fungal development in culture-media) aren't usable; in general they do contribute to some insight in (some combinations of) external (environmental) influences. They hint which factors in mould-research should be included.



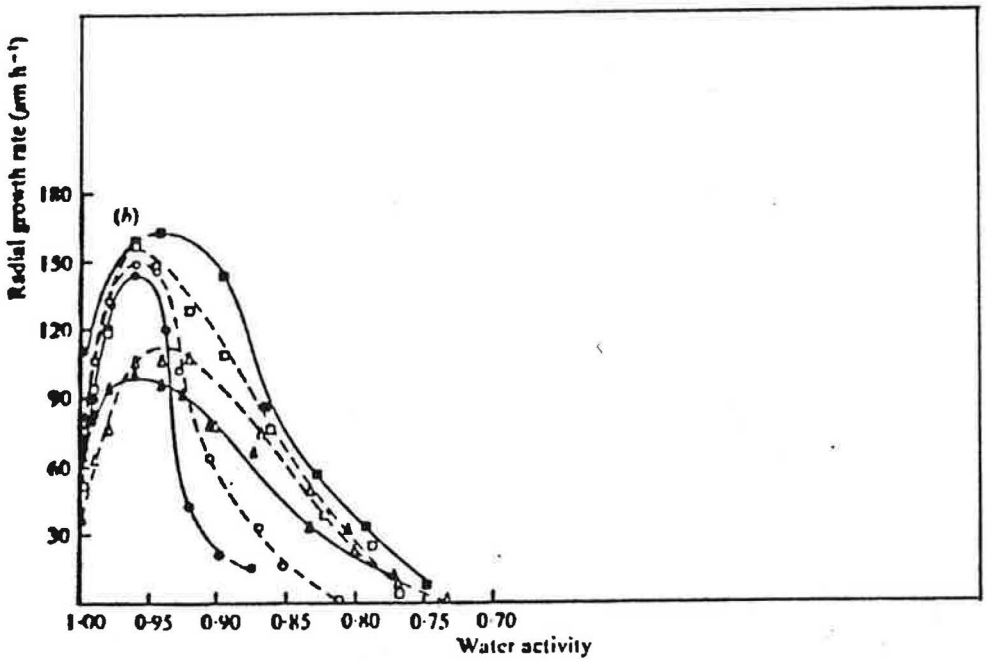
The effect of wateractivity and temperature on germination of Aspergillus restrictus. The numbers in the figure show the germination-period in days. The black spots represent conditions where no germination has been established after 95 days (Smith,Hill,'82)



The effect of temperature and wateractivity on the growth-rate of Aspergillus restrictus. The numbers in the figure represent growth-rates in mm/day. (Smith, Hill, '82)

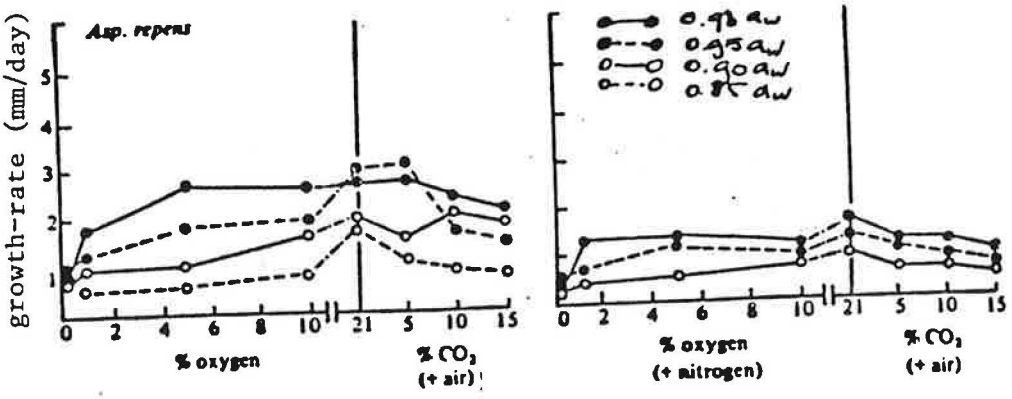


Chemical change of a substrate (Aspergillus oryzae is present in the substrate) (Cochrane,'58)



(●) NaCl, pH 4·0; (○) NaCl, pH 6·5; (■) glucose/fructose, pH 4·0; (□) glucose/fructose, pH 6·5; (▲) glycerol, pH 4·0; (△) glycerol, pH 6·5.

The effect of wateractivity,and acidity and solution on the growth-rate of Eurotium-chevalieri (Pitt,Hocking,'77)



The effect of wateractivity, O_2 and CO_2-concentrations and temperature on growth of Aspergillus repens (Magan&Lacey,'84)