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STANDARD

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**Optics and optical instruments —
Environmental test methods —**

Part 11:
Mould growth

*Optique et instruments d'optique — Méthodes d'essais
d'environnement —*

Partie 11: Moisissures



Reference number
ISO 9022-11:1994(E)

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 9022-11 was prepared by Technical Committee ISO/TC 172, *Optics and optical instruments*, Subcommittee SC 1, *Fundamental standards*.

ISO 9022 consists of the following parts, under the general title *Optics and optical instruments — Environmental test methods*:

- Part 1: *Definitions, extent of testing*
- Part 2: *Cold, heat, humidity*
- Part 3: *Mechanical stress*
- Part 4: *Salt mist*
- Part 5: *Combined cold, low air pressure*
- Part 6: *Dust*
- Part 7: *Drip, rain*
- Part 8: *High pressure, low pressure, immersion*
- Part 9: *Solar radiation*
- Part 10: *Combined sinusoidal vibration, dry heat or cold*

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- *Part 11: Mould growth*
- *Part 12: Contamination*
- *Part 13: Combined shock, bump or free fall, dry heat or cold*
- *Part 14: Dew, hoarfrost, ice*
- *Part 15: Combined random vibration wide band: reproducibility medium, in dry heat or cold*
- *Part 16: Combined bounce or steady-state acceleration, in dry heat or cold*
- *Part 17: Combined contamination, solar radiation*
- *Part 18: Combined damp heat and low internal pressure*
- *Part 19: Temperature cycles combined with sinusoidal or random vibration*
- *Part 20: Humid atmosphere containing sulfur dioxide or hydrogen sulfide*

Annex A of this part of ISO 9022 is for information only.

Introduction

Optical instruments are affected during their use by a number of different environmental parameters which they are required to resist without significant reduction in performance.

The type and severity of these parameters depend on the conditions of use of the instrument (for example, in the laboratory or workshop) and on its geographical location. The environmental effects on optical instrument performance in the tropics and subtropics are totally different from those found when they are used in the arctic regions. Individual parameters cause a variety of different and overlapping effects on instrument performance.

The manufacturer attempts to ensure, and the user naturally expects, that instruments will resist the likely rigours of their environment throughout their life. This expectation can be assessed by exposure of the instrument to a range of simulated environmental parameters under controlled laboratory conditions. The severity of these conditions is often increased to obtain meaningful results in a relatively short period of time.

In order to allow assessment and comparison of the response of optical instruments to appropriate environmental conditions, ISO 9022 contains details of a number of laboratory tests which reliably simulate a variety of different environments. The tests are based largely on IEC standards, modified where necessary to take into account features special to optical instruments.

It should be noted that, as a result of continuous progress in all fields, optical instruments are no longer only precision-engineered optical products, but, depending on their range of application, also contain additional assemblies from other fields. For this reason, the principal function of the instrument must be assessed to determine which International Standard should be used for testing. If the optical function is of primary importance, then ISO 9022 is applicable, but if other functions take precedence then the appropriate International Standard in the field concerned should be applied. Cases may arise where application of both ISO 9022 and other appropriate International Standards will be necessary.

Optics and optical instruments — Environmental test methods —

Part 11: Mould growth

WARNING — Although the species of fungi selected for testing do not normally present a hazard to humans, certain people may develop allergies or other reactions. The use of experienced and trained personnel is required to ensure the proper handling of fungi and the appropriate conduct of the tests. It is, therefore, recommended to entrust the performance of the tests required by this part of ISO 9022 to a microbiological laboratory, since such laboratories have the appropriate equipment and trained personnel.

1 Scope

This part of ISO 9022 specifies methods for the testing of optical systems, instruments containing optical components, and materials for optical instruments, under equivalent conditions, for their ability to resist mould growth.

However, complete instruments or assemblies are only tested as specified in this part of ISO 9022 in exceptional cases. Normally, representative specimens such as mounted optics, material samples, or surface coatings on representative samples are used for testing.

The tests described are designed for the selection of materials and components for instruments likely to be used in an environment that is conducive to mould growth, rather than for regular production control.

The purpose of testing is to investigate to what extent the optical, chemical, mechanical and electrical performance characteristics of the specimen are affected by mould growth.

In addition, the tests are designed to assess to what extent metabolic waste products (such as enzymes or acids), excreted by fungi, cause etching, corrosion, or short-circuits on, for instance, printed circuit boards.

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this part of ISO 9022. At the time of publication, the edition indicated was valid. All standards are subject to revision, and parties to agreements based on this part of ISO 9022 are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 9022-1:1994¹⁾, *Optics and optical instruments — Environmental test methods — Part 1: Definitions, extent of testing.*

1) To be published.

3 General information and test conditions

3.1 Test fungi

The species of fungi selected for testing (see table 1) are frequently found on optical glass surfaces. Among them are species of hydrophyl, mesophyl and xerophyl fungi.

Table 1 — Test fungi

Series No.	Species
1	<i>Aspergillus niger</i>
2	<i>Aspergillus flavus</i>
3	<i>Aspergillus versicolor</i>
4	<i>Trichoderma viride</i>
5	<i>Penicillium funiculosum</i>
6	<i>Penicillium citrinum</i>
7	<i>Paecilomyces</i>
8	<i>Chaetomium globosum</i>
9	<i>Eurotium tonophilum</i>
10	<i>Aspergillus penicilloiden (Vitrocolae)</i>

Since strains of fungi change their characteristics with time, only fungal species are specified. The test report, or relevant specification respectively, shall however specify the fungal strains used for testing.

3.2 Fungal spore suspension

3.2.1 Fungal cultures

Pure cultures of each of the fungus species specified in table 1 shall be maintained separately on an appropriate agar medium (e.g. malt agar).

The fungal cultures used for the spore suspension shall not be older than 14 days to 21 days and shall not be used more than once for preparing a mixed spore suspension.

3.2.2 Spore suspensions

For preparing the spore suspensions, and wherever else in this subclause "water" is specified, use distilled or fully demineralized sterile water containing 0,05 % (m/m) of a non-toxic wetting agent such as sodium dioctylsulfosuccinate or sodium laurylsulfate.

Pour 10 ml of the water into each of the fungal cultures described in 3.2.1.

Using a sterile platinum loop, or any other suitable means, carefully scrape the spores from the mycelial mat. Take care to leave out clumps of agar. Pour the spores charge into a sterile Erlenmeyer flask containing 45 ml of water. Add sterile solid glass beads and shake vigorously to liberate the spores from the fruiting body and to break the spore clumps. Filter the dispersed fungal spore suspension through sterile glass wool to remove mycelial fragments.

Centrifuge the filtrate and discard the supernatant liquid. Resuspend the residue in 50 ml of water and centrifuge. Wash the spores obtained from each of the fungi in this manner three times.

Dilute the final washed residue with the mineral salts solution specified in table 2 in such a manner that each resultant spore suspension contains $(1\ 000\ 000 \pm 200\ 000)$ spores per millilitre, measured using a suitable counting chamber.

Table 2 — Mineral salts solution

Component	Mass g
Potassium dihydrogen orthophosphate (KH_2PO_4)	0,7
Potassium monohydrogen orthophosphate (K_2HPO_4)	0,7
Magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	0,7
Ammonium nitrate (NH_4NO_3)	1,0
Sodium chloride (NaCl)	0,005
Iron(II) sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)	0,002
Zinc sulfate heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$)	0,002
Manganese(II) sulfate monohydrate ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$)	0,001
Distilled water (H_2O)	1 000,0

Sterilize the mineral salts solution in an autoclave at 120 °C for 20 min. Using sodium hydroxide solution, $c(\text{NaOH}) = 0,01$ mol/l, adjust the pH of the solution to 6,0 to 6,5 after sterilization. (Percentage purity of the chemicals: atomic adsorption spectroscopy.)

Inoculate each of ten Petri dishes containing an appropriate agar medium (e.g. malt agar) with spore suspension and immediately incubate the dishes, to check the viability of each fungus species, in the incubation chamber to be used for exposing the specimens. In the event that fungicide-treated specimens are under test in the incubation chamber, expose the Petri dishes to exactly the same climatic conditions in a separate incubation chamber. The absence of

growth of any of the various fungus species, at the end of one week, will invalidate the results of all simultaneously performed tests using these spores. Such invalidated tests shall be repeated using freshly prepared mixed spore suspensions from new cultures.

3.2.3 Mixed spore suspension

After having taken the inoculum from the spore suspensions for the purpose described in 3.2.2, blend equal portions of the ten spore suspensions to obtain the final mixed spore suspension.

The spore suspensions from the individual cultures as well as the mixed spore suspension shall be used on the day of their preparation. On no account shall they be stored for later use.

3.3 Control strips

Along with the specimens place at least three control strips in the exposure chamber in order to ensure that optimal climatic conditions are present in the incubation or climatic chamber during exposure of the contaminated specimens. The control strips are of no use if the specimens have been previously treated with fungicides: since these become active predominantly during the volatile phase, a fungicidal atmosphere would develop within the test chamber and hamper the fungal growth on the control strips. In such cases, only the separately incubated individual fungal cultures may be used as control.

The control strips shall be of white sterilized filter paper and shall be of the same size as the specimen (see 3.4). Dip the control strips into the nutrient solution specified in table 3 and hang them to dry in a sterile atmosphere. Freshly prepare the nutrient solution immediately before impregnating the control strips. Use the control strips on the day of their preparation.

Using hydrochloric acid (HCl) or sodium hydroxide (NaOH) solution, adjust the pH of the solution to 5,3.

3.4 Specimen

Unless the testing of complete instruments or assemblies is required in the relevant specification, representative samples shall be used for testing. Specimens of the size and dimensions show in figure 1 and of at least 1 mm thickness should preferably be used as representative samples of non-metallic coatings or lubricants to be tested.

Table 3 — Nutrient solution for soaking control strips

Component	Mass g
Potassium dihydrogen orthophosphate (KH ₂ PO ₄)	0,1
Ammonium nitrate (NH ₄ NO ₃)	0,1
Magnesium sulfate heptahydrate (MgSO ₄ .7H ₂ O)	0,025
Yeast extract	0,05
Glycerol [C ₃ H ₅ (OH) ₃]	10,0
Distilled water (H ₂ O)	90,0

NOTE 1 Sample sheets of 140 mm ± 2 mm or 280 mm ± 2 mm in length may also be indicated in the appropriate specification.

The coating under test (e.g. varnish) shall be of the same structure as the coating intended for the instrument or for parts of the instrument.

Prior to applying the coating, prepare the surface of the specimen in the same manner as required for the original instrument. The coating shall completely surround the specimen so as to cover particularly edges, corners and the edges of holes. The coating shall not be degraded by identification marking; numbers, etc. shall be punched prior to applying the coating.

Sterilize specimens at a temperature of 180 °C to 200 °C before applying lubricants as a thin film on one side only.

Use glass hooks or polyamide threads for suspending the specimens in the exposure chamber.

3.5 Incubation cabinets and climatic chambers

The specimens inoculated with the mixed spore suspension shall be incubated in incubation cabinets or climatic chambers under the climatic conditions specified in clause 4.

The incubation cabinets or climatic chambers shall be adjustable to 29 °C ± 1 °C and the temperature may vary in time by ± 0,5 K per hour. The cabinets or chambers shall be moisture-proof and shall be provided with a pressure-relief capillary tube or a valve in order to prevent, during exposure, the build-up of pressure within the test chamber.

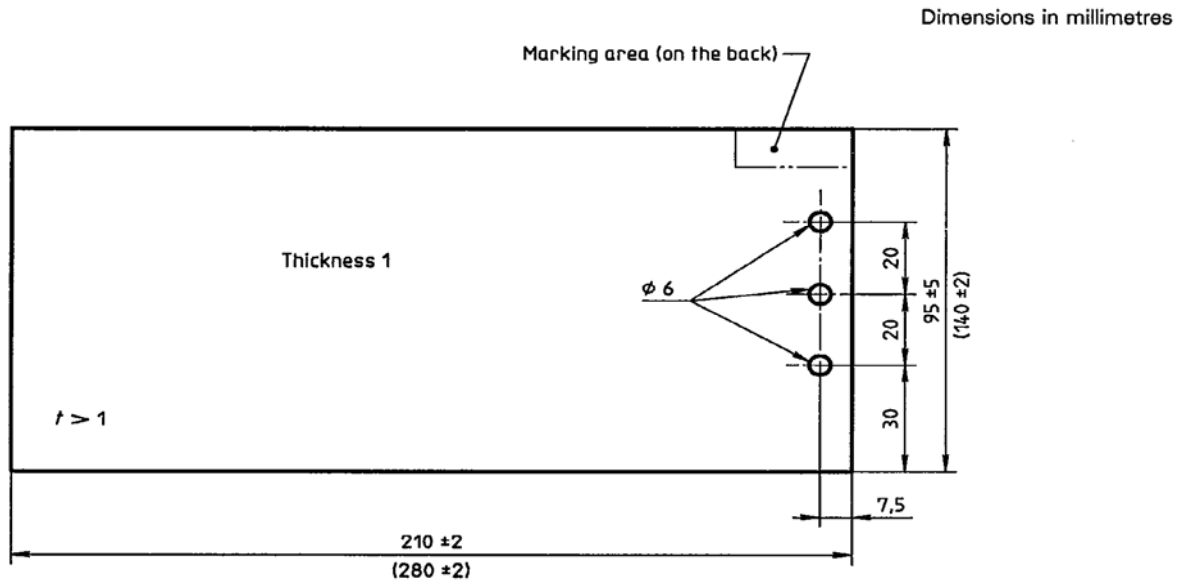


Figure 1 — Sample sheet

Normally, there shall be no air circulation during exposure. In the event that the relevant specification does require air circulation, the air flow velocity shall not exceed 0,5 m/s.

Use distilled, demineralized or deionized water for humidification. A saturated solution (with an ample solid phase) of potassium sulfate (K_2SO_4), covering the entire bottom of the test chamber, may be used to maintain the required relative humidity, which will reach 96 % above the solution.

Moisture shall not be allowed to condense on the specimens during exposure in order to prevent the mixed spore suspension applied to the specimens from being washed off. For the same reason, the specimens shall be protected from condensed-moisture drops falling from the chamber walls.

The contact areas of fixtures supporting the specimen shall be kept to a minimum.

The size of the test chamber and the arrangement of the specimens shall be selected so as to ensure uniform conditioning and aeration of all specimens.

If it is necessary continuously to record the test chamber temperature and relative humidity during exposure, a method shall be specified in the relevant specification.

4 Conditioning method 85: Mould growth

See table 4.

Table 4 — Degrees of severity for conditioning method 85: Mould growth

Degree of severity	01	02
Exposure time	28	84
Temperature °C	29 ± 1	
Relative humidity %	96 ± 2	
Number of spores per cm ² of specimen surface	15 000 ± 3 000	
State of operation	1	

5 Procedure

5.1 General

The test shall be conducted in accordance with the requirements of the relevant specification and ISO 9022-1.

5.2 Preconditioning

Unless otherwise specified in the relevant specification, use water containing a wetting agent (in accordance with 3.2.2) to clean the specimens, then hang them to dry. Care shall be taken during cleaning not to leave any fragments of the cleaning material (such as cloth or cottonwool) on the specimen. Prior to and during testing, handle the specimens so as not to contaminate them by fingerprints or in any other way. Apply lubricants to the specimens (see 3.4) immediately before exposure.

Then inoculate the specimens and at least three control strips (3.3) with the mixed suspension (see 3.2.3) by spraying the suspension on the specimens and control strips with an atomizer ensuring a uniform distribution of $(15\ 000 \pm 3\ 000)$ spores per cm^2 of the surface of the specimens.

Place the specimens and the control strips in the incubation cabinet or conditioning chamber not later than 15 min after inoculation. The test chamber shall have been operated at the required climatic conditions at least for 4 h before starting the incubation of the specimens.

In the event that the relevant specification not only requires the evaluation of the mould growth but also evaluation of potential corrosion caused by the mould growth and measurement of the transmission of optical instruments, as many identical non-inoculated specimens as inoculated specimens shall be exposed to identical climatic conditions in separate incubation cabinets or climatic chambers. This is the only known method by which it is possible to distinguish between damage caused by mould growth and damage caused by climatic stress.

5.3 Activities during conditioning

Inspect control strips and Petri dishes for mould growth after seven days of conditioning in accordance with clause 4. If there is little or no growth on the control strips and/or the Petri dishes, the entire test is unacceptable and shall be repeated.

During conditioning, open the incubation cabinet or conditioning chamber once a week for a few seconds, for the purpose of air renewal. At the end of the exposure time, the mould growth on the control strips shall be more substantial in density than on the seventh day of conditioning; otherwise, the test is unacceptable and shall be repeated.

5.4 Recovery

Unless otherwise specified in the relevant specification, evaluate the specimens at the end of the conditioning period without leaving them to dry first. On no account shall specimens be cleaned prior to evaluating the mould growth. If a comparative corrosion evaluation is required, carefully remove the mycelia, using water and a soft cloth, after the evaluation of the mould growth.

5.5 Evaluation

For the evaluation of the extent of mould growth, refer to table 5. If, in addition to mould growth evaluation, evaluation of corrosion damage due to mould growth is required, remove the mycelia and compare the specimens with the non-inoculated specimens.

5.6 General level for acceptance

Unless otherwise specified in the relevant specification, the test shall be accepted as successful if the amount of growth is less than or equal to grade 2 in table 5.

Table 5 — Evaluation of the extent of mould growth

Amount of growth grade	Surface of substrate covered %	Extent of growth
0 ¹⁾	0	Substrate is devoid of mould growth
1 ¹⁾	more than 0 to 10	Sparse or very restricted mould growth (traces)
2	more than 10 to 30	Intermittent or loosely spread mould colonies on substrate surface (visible with the naked eye)
3	more than 30 to 70	Substantial amount of mould growth (easily visible)
4	more than 70	Massive mould growth

1) Use $\times 50$ magnification with appropriate illumination when evaluating these grades.

6 Environmental test code

The environmental test code shall be as specified in ISO 9022-1.

EXAMPLE

The environmental test of material for optical instruments for resistance to mould growth, conditioning method 85, degree of severity 01, state of operation 1, shall be identified as:

Environmental test ISO 9022-85-01-1

7 Specification

The relevant specification shall contain the following details:

- a) environmental test code;
- b) number of specimens;
- c) type and dimensions of specimens;
- d) arrangement and position of the specimens in the conditioning cabinet or climatic chamber;
- e) where appropriate: identification of the fungal strains of the species of fungi listed in table 1;
- f) number and type of non-contaminated specimens;
- g) requirements regarding air circulation in the conditioning chamber;
- h) requirements with respect to recording the temperature and relative humidity;
- i) type and scope of initial test;
- j) preconditioning if other than specified in 5.2;
- k) recovery if other than specified in 5.4;
- l) type and scope of final test;
- m) criteria for evaluation, taking into account 5.5;
- n) type and scope of test report.

Annex A (informative)

Explanatory notes

Fungal spores are omnipresent. Provided that the environmental conditions, such as humidity and temperature, are favourable, the spores will germinate without needing extraneous nutrients. The majority of fungi prefer temperatures between 20 °C and 30 °C and a relative humidity of 90 % to 100 % as an optimal environment for germination and further growth. Due to the presence of nutrients adsorbing or absorbing humidity, such conditions may even develop, in the form of a microclimate, immediately above the surface of substrates without the required growth-stimulating conditions being present in the microclimatic environments.

After germination, nutritious substances are required for further growth. Minute amounts of organic matter, such as textile fibres, traces of organic fats, varnishes, fingerprints, dust and other organic contaminants will be enough to stimulate growth. The germinal tube starts secreting enzymes required to process the available nutritious matter for further growth and development of the mycelium.

Mould can germinate, but not grow, even on clean inorganic material surfaces such as, for instance, silicate lenses as used in optical instruments. For this reason, optical lenses should not be tested for mould growth unless they are an integral part of an instrument to be tested, although it is the mould growth on optical surfaces that, in fact, causes functional degradation of optical instruments. The reason for mould growth on mounted lenses is the fact that traces of organic contamination on the optical surfaces serve the spores as food stock, and that nutrient organic material is deposited in the joint between the cell and the lens during the process of cleaning the optical surfaces.

Cell elements coated with inadequate varnish or made of synthetic material may also serve as a food source for fungi. It is mainly from these peripheral zones that the mycelium will spread its typical fine-veined or lichenous network across the lens surface. Unless removed at an early stage and depending on the type of glass used, more or less severe irreversible etchings of the lens surface may be left behind in the shape of a "print" of the mycelial network. These

etchings are chiefly brought about by the acid metabolic products of the fungi.

The etching effect of the metabolic products is presumably supported by the increased moisture concentration in the immediate vicinity of the mycelium. The varied susceptibility of different types of glass to mould infestation and resultant etching finds its explanation in the attacking mechanism described above. Thus, mould growth can do no permanent damage to fused silica because of the latter's resistance to acids (except for hydrofluoric acid) and water. Although glass types of a satisfactory resistance to atmospheric corrosion and acids show but poor inhibitory action towards mould growth, little or no permanent damage will be observed after removal of the mycelium. Glass types of a poor resistance to atmospheric corrosion and acids will, however, show clear traces of etching even after a short time of exposure to mould growth. On the other hand, the expansion of the mycelium across the lens surface is inhibited by the release of toxic substances from the glass during the process of etching.

Blooming coatings cause little or no change in the mechanisms of mould growth and etching as far as the type of glass is concerned. However, the increased contrast capability of coated lenses permits detection even of isolated hyphae (mycelial threads) of a size which would not be visible to the naked eye.

So far, there is no way of efficiently protecting optical surfaces by means of chemical substances (fungicides) that inhibit or prevent mould growth. The application of fungicides to the surface of optical lenses would substantially degrade their optical transmission. Some temporary protective effect on mould growth from the peripheral joint may be achieved by incorporating into the edge of the lens or the cell varnish fungicides that become active during their volatile phase. Such fungicides are able to poison the nutrients settling in the joint between the cell and the lens. An essential and most effective way of fighting mould infestation is to clean frequently and thoroughly all accessible lens surfaces.

As far as encased optical surfaces, especially in properly sealed internal compartments, are concerned, a

relatively satisfactory, albeit temporary, protection from mould infestation can be achieved by employing fungicides. Care should however be taken to ensure that spores enclosed during instrument assembly are destroyed to as large an extent as possible.

Furthermore, desiccator cartridges may be placed in internal compartments in order to keep the relative humidity below 65 %, thus preventing fungi from germinating and growing.

Materials of an organic nature, such as leather, paper and other cellulose derivatives, textiles, felt, oil varnishes, and vegetable and animal oils and fats, are most susceptible to mould growth.

Unplasticized synthetic material with or without inorganic fillers is fairly unlikely to be infested, while such material, if plasticized or containing organic fillers, is susceptible to mould infestation, the grade of susceptibility depending upon the type of plasticizer used. Derivatives of fatty acids, for instance, are particularly conducive to mould growth whereas those of phthalic acids are less stimulating.

When selecting fungicides for incorporation into materials or for impregnation of materials and varnishes,

the following points should be taken into consideration.

- a) The volatilization pressure of the fungicide, at ambient temperature, shall be high enough to maintain anywhere within the instrument a concentration sufficient to prevent mould growth; however, condensation of the fungicide on optical surfaces, or corrosion of components caused by the fungicide, is unacceptable.
- b) Operational and testing temperatures of the instrument shall not degrade the stability of the fungicide.
- c) The fungicide shall not be soluble in water.
- d) The volatilization pressure of the fungicide shall be standardized to be effective over an optimal period.
- e) Fungi may become resistant to a fungicide attacking them over a prolonged period.
- f) The effectiveness of fungicides used in compartments that are not absolutely airtight will be limited in time.

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