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WORKING PAPER ON DRAFT TEST GUIDELINES FOR MUSHROOMS

*(Agaricus spec. L.)*

*Document prepared by experts from the Netherlands*

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I. Subject of these Guidelines

These Test Guidelines apply to all varieties of *Agaricus bisporus* L., *Agaricus bitorquis* L. and *Agaricus arvensis* L. (*Agaricaceae*).

II. Material Required

1. The competent authorities decide when, where and in what quantity and quality the plant material required for testing the variety is to be delivered. Applicants submitting material from a State other than that in which the testing takes place must make sure that all customs formalities are complied with. As a minimum, for each year of test the following quantity of material is recommended:

1 liter of spawn  
(on cooked cereal grains; and as specified by the testing authority)

The quality of the material to be delivered should not be below the standards of commercial spawn for marketing in the country concerned, especially in regard to quantity of hyphae. Mycelium on grain should be visible to the naked eye; the grain should not be colonized to such an extent that kernels stick together. The spawn should not be older than 6 months and having been stored under proper conditions (i.e. 2-4 °C).

2. The spawn must not have undergone any treatment unless the competent authorities allow or request such treatment. If it has been treated, full details of the treatment must be given.

III. Conduct of Tests

1. The minimum duration of tests should normally be two independent growing cycles of three flushes each.

2. The tests should normally be conducted at one place. If any important characteristics of the variety cannot be seen at that place, the variety may be tested at an additional place.

3. The tests should be carried out under conditions ensuring normal growth. The size of the plots should be such that fruit bodies or parts of the fruit bodies may be removed for measurement and counting without prejudice to the observations which must be made up to the end of the growing period. As a minimum, each test should include at least a total of **180** fruiting bodies, which should be divided between 6 replicates. Separate plots for observation and for measuring can only be used if they have been subject to similar environmental conditions.

4. Additional tests for special purposes may be established.

#### IV. Methods and Observations

1. All observations determined by measuring or counting should be made on 30 fruit bodies or parts of 30 fruit bodies per replicate.
2. Unless otherwise indicated, all characteristics of the fruit bodies, the cap, the stipe and the gills (characteristics 2 to 18 and 22 to 25) should be recorded at harvest maturity (button stage 1, 2 and 3 [see annex page ....] hand picked mushrooms; freshly harvested). The characteristics of the open cap should be recorded as soon as the cap is fully spread (and not postponed until later date) (characteristics 19 to 21). Records should preferably be made from first and second flush; the third flush may give some additional information.
3. When disease resistance characteristics are used for assessing distinctness, uniformity and stability, records must be taken under conditions of controlled infection with a defined pathotype. If applicable, all resistances should be tested separately on each race and separately for each pathogen.
4. For the assessment of uniformity a population standard of 1% with an acceptance probability of at least 95% should be applied. In case of a sample size of 180 mushrooms the maximum number of off-types allowed would be 4.
5. Unless otherwise indicated, all example varieties mentioned in the Table of Characteristics represent the corresponding state of expression under standardized growing conditions. The variety descriptions preferably should, especially in case of this crop, state the standardized growing conditions, such as temperature, CO<sub>2</sub>-level and relative humidity conditions, as well as the cultivation system.

#### V. Grouping of Varieties

1. The collection to be grown should be divided into groups to facilitate the assessment of distinctness.
2. Suitable characteristics for grouping purposes are those which are known from experience not to vary, or to vary only slightly, within a variety and which in their various states are fairly evenly distributed within the collection.
4. It is recommended that the competent authorities use the following characteristics for grouping varieties:
  - (a) Stipe: shape (in longitudinal section) (characteristic 5)
  - (b) Cap: shape (in longitudinal section) (characteristic 12)
  - (c) Cap: color (characteristic 15)
  - (d) Cap: central part of upper side (characteristic 17)

VI. Characteristics and Symbols

1. To assess distinctness, uniformity and stability, the characteristics and their states as given in the Table of Characteristics should be used.

2. Notes (1 to 9), for the purposes of electronic data processing, are given opposite the states of the different characteristics.

3. Legend:

(\*) Characteristics that should be used every growing period for the examinations of all varieties and should always be included in the description of the variety, except when the state of expression of a preceding characteristic or regional environmental conditions render this impossible.

(+) See Explanations on the Table of Characteristics in Chapter VIII.

VII. Table of Characteristics/Tableau des caractères/Merkmalstabelle/Tabla de caracteres

English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<b>1. Basidium: number</b>					
<b>(*) of spores</b>					
<b>(+)</b>					
two				Horronda, Horwitu	2
between 2 and 4					3
four				Horbita, Horvensis	4
<b>2. Stipe: length</b>					
<b>(+)</b>					
short				Horwitu	3
medium				Le Lion B86, Somycel 76	5
long				Somycel 53	7
<b>3. Stipe: diameter</b>					
<b>(+)</b>					
small				Somycel 91	3
medium				Somycel 76	5
large				Horronda, Horwitu	7
<b>4. Stipe: ratio length/diameter</b>					
small					3
medium					5
large					7
<b>5. Stipe: shape</b>					
<b>(*) (in longitudinal section)</b>					
<b>(+)</b>					
cylindrical				Horronda, Horvensis	1
obconical / tapered				Horwitu	2
swollen base				Horbita	3

English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<b>6. Stipe: distance from base to veil remnant ring</b>					
(+)					
short				Commissaris Cremers	3
medium				Horbita	5
long				Horvensis	7
<b>7. Stipe: structure (in longitudinal section)</b>					
	<i>delete ? (A.S.)</i>				
hollow					3
partly hollow					5
not hollow					7
<b>8. Stipe: firmness</b>					
	<i>delete ? (A.S.)</i>				
soft					3
medium					5
firm					7
<b>9. Cap: height</b>					
(+)					
short					3
medium					5
tall					7
<b>10. Cap: diameter</b>					
(+)					
small				Commissaris Cremers	3
medium				Somycel 76	5
large				Horronda	7

English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<b>11. Cap: ratio height/diameter</b>					
small					3
medium					5
large					7
<b>12. Cap: shape (* (+) (+) (in longitudinal section)</b>					
obovate				Horvensis	1
circular				Commissaris Cremers, Horronda	2
transverse elliptic				Horwitu	3
<b>13. Cap: thickness (+) (in longitudinal section)</b>					
thin				Le Lion B86, Somycel 76	3
medium				Horronda	5
thick				Commissaris Cremers	7
<b>14. Cap: amount of scales (+)</b>					
absent or very low				Somycel 91, Royal 70, Royal 75	1
low				Horronda, Le Lion X13, Royal 24A	3
medium				Horwitu	5
high				Somycel 76	7
very high					9



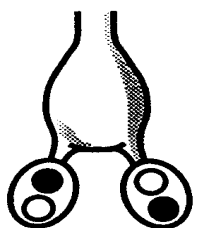
English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<b>15. Cap: color</b>					
(*)					
<i>Remark from F: Should this characteristics be measured very exact?</i>					
white				Royal 75, Somycel 91	1
off-white				Claron A3.01, Somycel 76	2
pale yellowish				Horvensis	3
brown				Le Lion C9	4
<b>16. Cap: firmness</b>					
<i>delete ? (A.S.)</i>					
soft					3
medium					5
firm					7
<b>17. Cap: central part of upper side</b>					
(*)					
(+)					
rounded					1
flat					2
depressed					3
<b>18. Gills: color(at time of breaking of the veil)</b>					
(+)					
pink					1
pale orange				Horvensis	2
light brown				Horronda, Horwitu	3
dark brown					4
<b>19. Stipe: aspect of veil remnant ring (cap fully spread)</b>					
(+)					
not frayed / smooth				Commissaris Cremers	3
partly frayed				Horbita, Claron VR77	5
frayed					7

English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<b>20. Cap: diameter (cap fully spread)</b> (+)	<i>delete ?</i>				
small				Le Lion X13, Royal 75	3
medium				Royal 20A	5
large				Somycel 76	7
<b>21. Cap: thickness (cap fully spread)</b> (+)					
thin					3
medium				Horwitu, Le Lion X13	5
thick				Claron A5.1, Somycel 205	7
<b>22. Cap: margin (cap fully spread)</b> (*)					
not frayed				Claron A5.1,Royal 26A	3
partly frayed				Horwitu,Somycel 205	5
frayed				Horronda	7
<b>23. Cap: central part of upper side (cap fully spread)</b> (*) (+)					
rounded					1
flat					2
depressed					3
<b>24. Fruiting body: weight</b>	<i>delete ?</i>				
low				Royal 75, Somycel 53	3
medium				Royal 20A	5
high				Le lion B62, Somycel 76	7

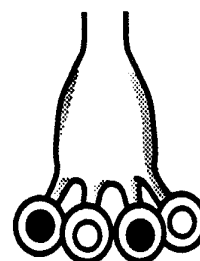
English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
<b>25. Discoloration of cutting surface</b>					
weak				Commissaris Cremers	3
medium				Horbita	5
strong					7
<b>26. Flushing pattern: earliness of first flush (*)</b>					
early				Le Lion X13, Horwitu	3
medium				Claron A5.1, Royal 26A	5
late				Le Lion X20, Somycel 205	7
<b>27. Flushing pattern: duration of first flush</b>					
short					3
medium					5
long					7
<b>28. Flushing pattern: earliness of second flush (*)</b>					
early					3
medium					5
late					7
<b>29. Flushing pattern: duration of second flush</b>					
short					3
medium					5
long					7

VIII. Explanations on the Table of Characteristics

Ad. 1: Basidium: number of spores

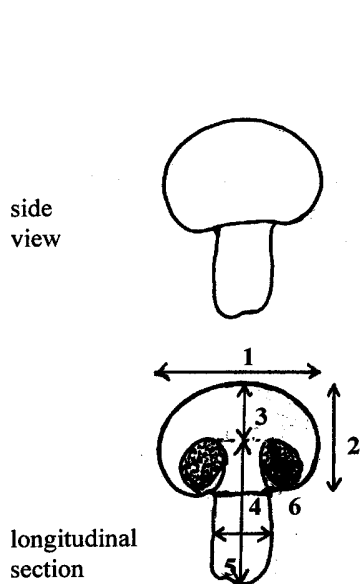


2  
two spores

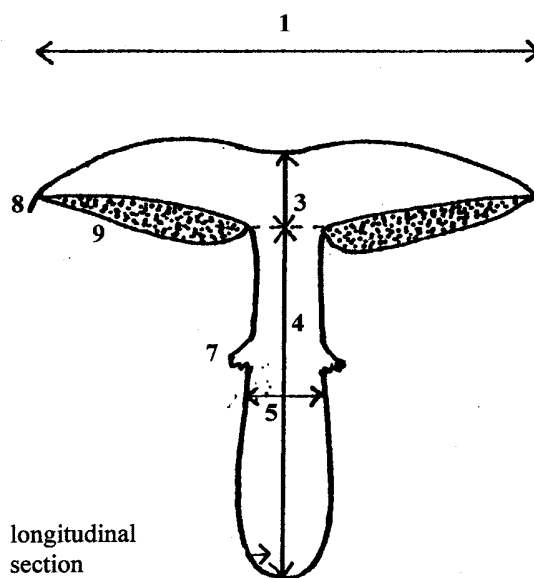


4  
four spores

Ad. 2, 3, 6, 9, 10, 13, 19, 20, 21 and 22: Mushroom: side view and longitudinal sections



**BUTTON**



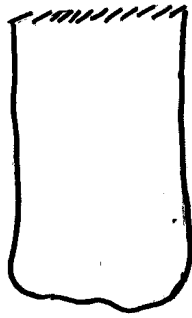
**FLAT / FULLY SPREAD**

Explanation:

- 1 - cap diameter
- 2 - cap height
- 3 - cap thickness
- 4 - stipe length
- 5 - stipe diameter

- 6 - veil
- 7 - veil remnant ring
- 8 - cap border
- 9 - gills

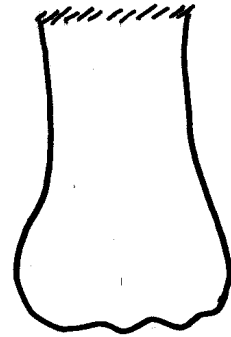
Ad. 5: Stipe: longitudinal section



1  
cylindrical

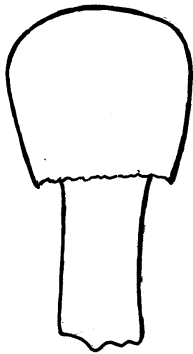


2  
obconical

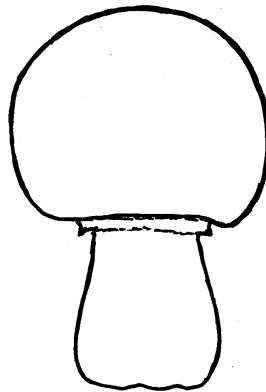


3  
swollen base

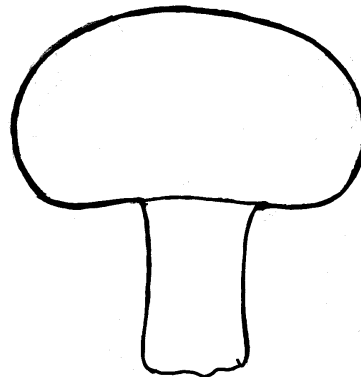
Ad. 12: Cap: shape (in longitudinal section)



1  
obovate

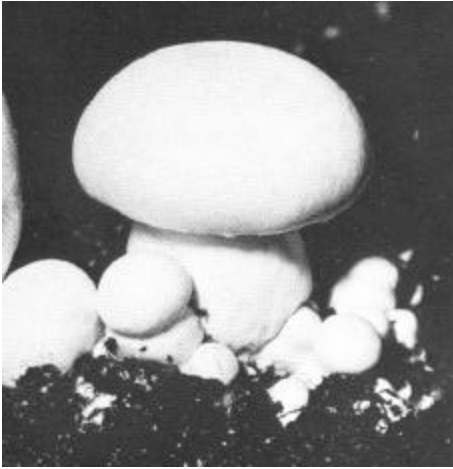


2  
circular



3  
transverse elliptical

Ad. 14: Cap: amount of scales



1  
absent or very low



9  
very high

Ad. 17 and 23: Cap: central part of upper side



1  
rounded

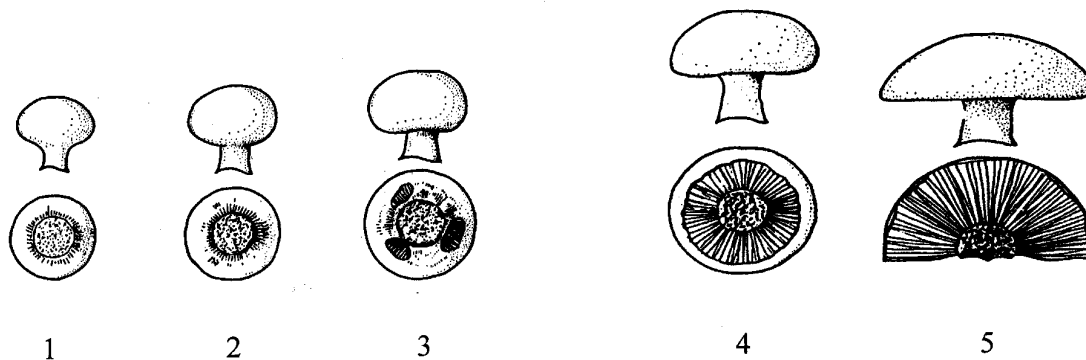


2  
flat



9  
depressed

Ad. 18 and 22: Veil and Gills: (from below)

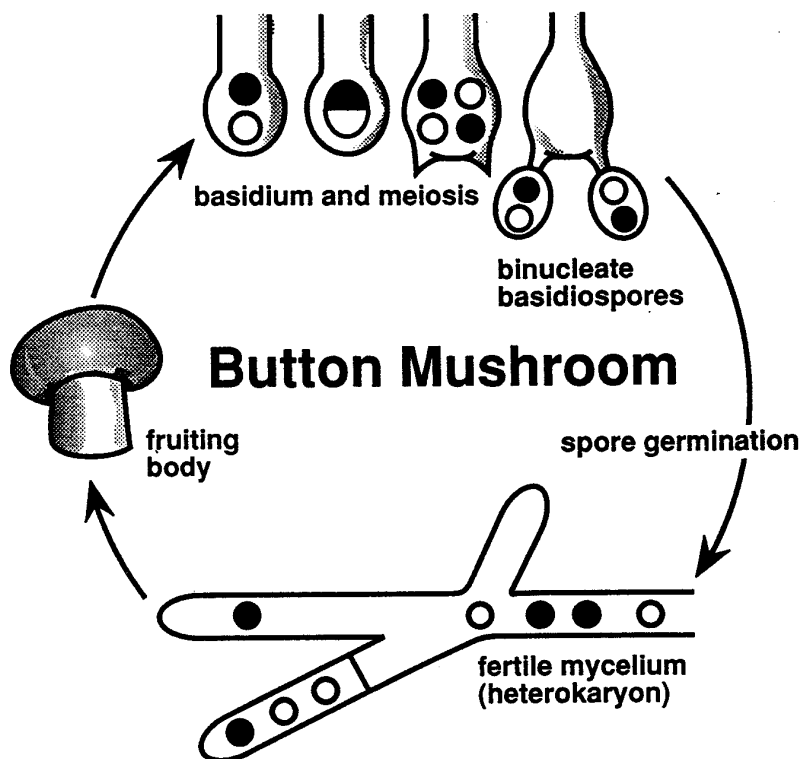


**Explanation:**

1, 2 and 3	- button stage	4	- opening / gills visible
1 and 2	- veil closed	5	- fully open / flat stage
3	- veil breaking		

Additional information:

Life cycle of *Agaricus bisporus*



IX. Literature

- FLEGG, P.B., SPENCER, D.M. and WOOD, D.A., 1985: The Biology and Technology of the Cultivated Mushroom, J. Wiley & Son, p. 347.
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- NEUT, A. van der, 1991: The development of a set of characteristics for DUS Tests of cultivated mushroom varieties, In: Genetics and Breeding of *Agaricus*, Pudoc Wageningen, Pp. 153-160
- SINGER, R., 1986: The *Agaricales* in Modern Taxonomy, Koeltz (Ger.), 981 pp and 80 pl.
- VOOREN, J.G. van de, POLDER, G. & HEIJDEN, G.W.A.M. van der, 1991: Application of image analysis for variety testing of mushroom, *Euphytica*, p. 57: 245-250
- VOOREN, J.G. van de, POLDER, G. & HEIJDEN, G.W.A.M. van der, 1992: Identification of Mushroom Cultivars Using Image Analysis, Transactions of the ASAE, p. 35-1: 347-350.



X. Technical Questionnaire

	Reference Number (not to be filled in by the applicant)
<p>TECHNICAL QUESTIONNAIRE to be completed in connection with an application for plant breeders' rights</p>	
1. Species	<p><i>Agaricus spec. L.</i>  MUSHROOM</p>
2. Applicant (Name and address)	
3. Proposed denomination or breeder's reference	
4. Information on origin, maintenance and reproduction of the variety	<p>4.1 method of maintenance and reproduction</p> <p>4.2 Other information</p>

5. Characteristics of the variety to be indicated (the number in brackets refers to the corresponding characteristic in Test Guidelines; please mark the state of expression which best corresponds).

Characteristics	Example Varieties	Note
<b>5.1 Basidium: number of spores (1)</b>		
two	Horronda, Horwitu	2[ ]
four	Horbita, Horvensis	4[ ]
<b>5.2 Stipe: shape (in longitudinal section) (5)</b>		
cylindrical	Horronda, Horvensis	1[ ]
obconical or tapered	Horwitu	2[ ]
swollen base	Horbita	3[ ]
<b>5.3 Cap: shape (in longitudinal section) (12)</b>		
obovate	Horvensis	1[ ]
circular	Commissaris Cremers, Horronda	2[ ]
transverse elliptic	Horwitu	3[ ]
<b>5.4 Cap: color (15)</b>		
white	Royal 75, Somycel 91	1[ ]
off-white	Claron A3.01, Somycel 76	2[ ]
pale yellowish	Horvensis	3[ ]
brown	Le Lion C9	4[ ]
<b>5.5 Cap: central part of upper side (17)</b>		
rounded		1[ ]
flat		2[ ]
depressed		3[ ]

Characteristics	Example Varieties	Note	
<b>5.6 Cap: central part of upper side (cap fully spread) (23)</b>			
rounded		1[ ]	
flat		2[ ]	
depressed		3[ ]	
<b>5.7 Flushing pattern: earliness of first flush (26)</b>			
early		3[ ]	
medium		5[ ]	
late		7[ ]	
<b>6. Similar varieties and differences from these varieties</b>			
Denomination of similar variety	Characteristic in which the similar variety is different <sup>o)</sup>	State of expression of similar variety	State of expression of candidate variety

7. Additional information which may help to distinguish the variety

7.1 Resistance to pests and diseases

7.2 Special conditions for the examination of the variety

- (i) Type of culture
  - in conditioned cells
  - in caves
  - Others, please specify.....

- (ii) Special temperature and humidity conditions
  - please specify.....
  - .....

(iii) Other conditions

7.3 Other information

8. Authorization for release

- (a) Does the variety require prior authorization for release under legislation concerning the protection of the environment, human and animal health?

Yes  No

- (b) Has such authorization been obtained?

Yes  No

If the answer to that question is yes, please attach a copy of such an authorization.