CIMMYT

Seed Testing of Maize and Wheat

A Laboratory Guide

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Sustainable
Maize and Wheat
Systems for the Poor

Seed Testing of Maize and Wheat
A Laboratory Guide

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Foreword

CIMMYT aims to help the poor by increasing the productivity of resources committed to maize and wheat in developing countries, while protecting the environment, through agricultural research and in concert with national research systems.

Germplasm improvement continues to be CIMMYT's main line of work, responding to a predicted increase in demand for advanced germplasm products and for source populations containing special traits. For this reason, CIMMYT also serves as a world storage and trust facility for the genetic resources of maize and wheat.

CIMMYT's germplasm improvement programs rely heavily on the free international exchange of maize and wheat seed. All concerned institutions, cooperators, and regulating authorities must have confidence in the safety of both imported and exported seed to facilitate such exchange. Accordingly, CIMMYT is fully committed to maintaining fundamental health standards in its worldwide operations to protect the agriculture of cooperators and host countries.

The Seed Health Unit at CIMMYT carries out phytosanitary examination of maize and wheat seeds (the Center's two mandated crops), prior to their export, and examination of any imported seeds. These examinations are carried out in close collaboration with the plant quarantine authorities of the Government of Mexico.

The unrestricted movement and exchange of germplasm is vital to progress in crop improvement programmes, but this movement must not jeopardize crops by spreading pests or diseases. International exchange of disease-free germplasm is possible if safeguards are imposed during seed production and seed distribution. Quarantine stations receive seeds for certification from various sources including seed industries, growers, research stations, individual scientists, traders, etc. The risk of spreading seed-borne pathogens varies widely with seed source. Seed from scientists and well-equipped seed industries is usually monitored during its active growing season by a qualified pathologist, to reduce these risks.

The authors, during the course of their work in the Seed Health Unit at CIMMYT, have intercepted a number of seed-borne organisms, and recorded their characteristics for easy identification. This manual describes each organism recorded with a series of photographs and gives a quick clue to facilitate rapid identification of the organism. In addition, details on distribution, significance, quarantine, detection techniques and references are given.

It is hoped that this manual will provide useful information to agricultural scientists and seed quarantine agencies testing maize and wheat seeds for import and export, and will help to minimize the risk of spreading diseases that could reduce crop yields and limit the amount of food produced by farmers.

Timothy Reeves
Director General,
CIMMYT

Introduction

Seed-borne organisms are either transmitted by, or transported with seed and survive as spores or resting structures within and on the seed. Seed-transmitted fungi often produce infected plants, while seed-transported fungi are considered of less importance in disease dispersal. However, both can provide an avenue by which a pathogen can be introduced into an area from which it was originally absent, and are therefore important in plant quarantine and to plant pathologists. No distinction is made between seed-transmitted and seed-transported fungi in this publication.

This pictorial laboratory manual is designed to facilitate the identification of 64 seed-borne organisms of maize and wheat. Each organism is described with a series of photographs illustrating the type of colony on seed and characteristics of the organism under a microscope. A quick clue is illustrated and described to facilitate rapid identification of the organism. In addition, details on distribution, significance, quarantine, detection technique and references are given. Quarantine details given are those identified in the FAO Global Plant Quarantine Information System (1994). Regulatory Plant Protection Organizations (RPPO's) are: EPPO (Europe), NEPPO (Near East), APPPC (Asia and Pacific), IAPSC (Africa), and COSAVE (South America). The list number is given for RPPO's only: A1= not present in the region; and A2 = present in part of the region and of quarantine importance elsewhere. Individual countries have been mentioned where known, but these are very incomplete. For Mexico, USA and Canada it is important to keep the data for individual countries as NAPPO does not have any regional list of plant quarantine organisms. Additional details are also given where known from assignments within the CIMMYT Seed Health Unit.

Differences in colony characters, morphology, and ornamentation of fruiting bodies which can be seen under the stereoscopic microscope are used to differentiate among the various genera. Fungal characters visible under a compound microscope are used to identify species. If the presence of a seed-borne pathogen of quarantine importance is suspected, it is advisable to confirm the identity of the fungus with the help of professional mycologists, and to prove its pathogenicity.

A synoptic key is provided at the beginning of the manual to facilitate the identification of the organism by distinguishing characteristics of colour and texture of colony on seed, shape and size of spores, etc. Each organism has a key number for identification, which is included in each group of characteristics positively displayed by the organism.

International Seed Health Testing Methods are used to detect the presence of seed-borne organisms on seeds. It is recommended that when testing seeds for quarantine and phytosanitary certification, these standardized procedures should be adopted, as this will help to eliminate situations where examinations in a receiving country reveal discrepancies with health certificates accompanying the seeds. The procedures for the principal Standard International Seed Health Testing Methods are given in Annex A for both maize and wheat separately.

High quality seed should not only be free from seed-borne diseases but should also have high germination capacity and vigour. Procedures for seed viability, germination and vigour tests routinely used in a seed testing laboratory are given in Annex B for both maize and wheat separately.

List of Organisms

Hyphomycetes - *Fusarium / Microdochium*

1. *Fusarium avenaceum*
2. *Fusarium crookwellense*
3. *Fusarium culmorum*
4. *Fusarium equiseti*
5. *Fusarium graminearum*
6. *Fusarium moniliforme*
7. *Fusarium oxysporum*
8. *Fusarium poae*
9. *Fusarium sambucinum*
10. *Fusarium tricinctum*
11. *Microdochium dimerum*
12. *Microdochium nivale*

Hyphomycetes - *Bipolaris / Drechslera / Exserohilum*

13. *Bipolaris cynodontis*
14. *Bipolaris hawaiiensis*
15. *Bipolaris maydis*
16. *Bipolaris sorokiniana*
17. *Bipolaris spicifera*
18. *Bipolaris victoriae*
19. *Bipolaris zeicola*
20. *Curvularia* spp.
21. *Drechslera avenacea*
22. *Drechslera dematioidea*
23. *Exserohilum rostratum*
24. *Exserohilum turcicum*

Other Hyphomycetes

25. *Acremoniella* spp.
26. *Acremonium* spp.
27. *Alternaria* spp.
28. *Arthrinium* spp.
29. *Aspergillus flavus / Aspergillus parasiticus*
30. *Aspergillus niger*
31. *Botrytis* spp.
32. *Cladosporium* spp.
33. *Epicoccum* spp.
34. *Gonatobotrys* spp.
35. *Monilia* spp.
36. *Myrothecium* spp.
37. *Nigrospora* spp.
38. *Papulospora* spp.
39. *Penicillium* spp.
40. *Rhinotrichum* spp.
41. *Stachybotrys* spp.
42. *Stemphylium* spp.
43. *Torula* spp.
44. *Trichoderma* spp.
45. *Trichothecium roseum*
46. *Ulocladium* spp.
47. *Verticillium* spp.

Coelomycetes

48. *Lasiodiplodia* spp.
49. *Pestalotiopsis* spp.
50. *Phoma* spp.
51. *Septoria nodorum*
52. *Stenocarpella macrospora*
53. *Stenocarpella maydis*

Ascomycetes

54. *Chaetomium* spp.
55. *Claviceps* spp.
56. *Melanospora* spp.
57. *Sordaria* spp.

Ustilaginales

58. *Sporisorium reilianum*
59. *Tilletia caries / Tilletia laevis*
60. *Tilletia controversa*
61. *Tilletia indica*
62. *Ustilago maydis*
63. *Ustilago nuda*

Zygomycetes

64. *Rhizopus* spp.

Key to Identification of Organisms

The identification key is a synoptic one in which the characters used in distinguishing the organisms are grouped in a number of categories such as taxonomic position, colony, mycelium, spore and other characteristics. Within each of these categories all the related characters are listed; for example, for conidium, all criteria of the conidium, such as pigmentation, shape, septation, size, ornamentation, formation etc., are listed.

Each organism included in the key has a unique number that corresponds to the number of the organism in the manual. The organism key number is listed in each group of characteristics where the feature is positively shown by that organism.

The advantage of a synoptic key is that it can be entered at any point. It is quicker to enter at a point where there are only a few numbers among which one needs to choose. These numbers usually apply to unusual characteristics and organisms with such unusual or odd criteria are therefore easier to identify. For organisms which can be separated only by a combination of several characters, there is almost no substitute to the time-consuming procedure of starting at the beginning of the key and working through it until the organism is identified. Identification can be speeded up if distinctive characters are used to reduce the number of initial possibilities. For example, if the conidia have appendages or hyaline end cells, then, because such features are only found in a comparatively small number of organisms in the key, the options are reduced and identification is quicker.

A working example of these two approaches is provided by attempting to key out *Stenocarpella macrospora*. One has the option of starting with seed appearance and working slowly through the key, or selecting a distinctive character and trying a short cut.

The longer option starts with seed appearance. The first criterion is part/whole of seed replaced by spores or seed intact. Seeds infected with *Stenocarpella macrospora* remain intact so there are 57 organisms to be considered:

1 2 3 4 5 6 7 8 9
10 11 12 13 14 15 16 17 18
19 20 21 22 23 24 25 26 27
28 29 30 31 32 33 34 35 36
37 38 39 40 41 42 43 44 45
46 47 48 49 50 51 52 53 54
56 57 64

The second group of characters concerns the colony on seed, and the first criterion is colour of colony. The colony colour of *Stenocarpella macrospora* is grey so a number of organisms with different colours of colonies may be eliminated:

(1) (2) (3) (4) (5) (6) (7) (8) (9)
(10) (11) (12) 13 (14) (15) (16) 17 (18)
19 20 21 (22) (23) (24) (25) 26 27
28 (29) (30) 31 (32) (33) (34) (35) (36)
(37) (38) (39) (40) (41) (42) (43) (44) (45)
(46) 47 (48) (49) (50) 51 52 53 (54)
(56) 57 (64)

Stenocarpella macrospora has a loose to spreading colony on seed and a few organisms not included here can be eliminated:

13 (17) (19) 20 21 (26) 27 28 31
47 51 52 53 57

The development of mycelium in colonies of *Stenocarpella macrospora* is sparse to moderate and some of the remaining organisms do not fall in this category, so can be eliminated:

13 20 21 27 (28) 31 47 (51) 52 53 (57)

The spores of *Stenocarpella macrospora* appear wet in mass, so a few organisms with spores appearing dry in mass may be eliminated:

(13) (20) (21) (27) (31) 47 52 53

Stenocarpella macrospora spores are black in mass, and only 47 is not included here so it is eliminated:

(47) 52 53

This leaves 52 and 53. At this point the simplest option is to look up the descriptions of 52 and 53 to decide which best fits *Stenocarpella macrospora*. Alternatively, one can continue to look through the key at each characteristic until a character is found which eliminates either of the two numbers: 52 or 53.

Stenocarpella macrospora belongs to the taxonomic position of coelomycetes, but since 52 and 53 fall in this category neither can be eliminated here.

52 53

Fruiting structures of *Stenocarpella macrospora* are a) pynidia, b) spherical to flask-shaped pycnidia, and c) pycnidia without setae, but again both 52 and 53 fall in these three categories and neither can be eliminated here.

52 53

Both 52 and 53, like *Stenocarpella macrospora,* have a) brown spores, and b) spores with transverse cross walls (septa) so neither is removed by these two categories.

52 53

The spores of *Stenocarpella macrospora* have a variable number of septa between 1 - 3. Only organism 52 has this character and it proves to be *Stenocarpella macrospora*.

52 (53)

The correct identity of the organism has been arrived at in 13 steps by using the synoptic key in this way.

The shorter alternative is to select key characteristics and start with those, irrespective of where they are situated in the key. Two such characters for *Stenocarpella macrospora* are the presence of pycnidia and relatively long conidia.

Thus, commencing with the pycnidia type of fruiting structure, the possible organisms are:

48 50 51 52 53

Turning now to the length of conidia, in *Stenocarpella macrospora* they are usually at least 50 µm in length so would fall under the criterion length 51-80 µm. In this criterion the majority of organisms do not produce conidia within a pycnidia and are therefore eliminated:

(1) (4) (6) (7) (13) (16) (18) (19) (21) (23) (24) (27) (38) 52 (55)

This leaves 52 as the correct identification and this has been arrived at in 2 steps, a simpler process than starting at the beginning of the synoptic key.

SYNOPTIC KEY

1. *Fusarium avenaceum*
2. *Fusarium crookwellense*
3. *Fusarium culmorum*
4. *Fusarium equiseti*
5. *Fusarium graminearum*
6. *Fusarium moniliforme*
7. *Fusarium oxysporum*
8. *Fusarium poae*
9. *Fusarium sambucinum*
10. *Fusarium tricinctum*
11. *Microdochium dimerum*
12. *Microdochium nivale*
13. *Bipolaris cynodontis*
14. *Bipolaris hawaiiensis*
15. *Bipolaris maydis*
16. *Bipolaris sorokiniana*
17. *Bipolaris spicifera*
18. *Bipolaris victoriae*
19. *Bipolaris zeicola*
20. *Curvularia* spp.
21. *Drechslera avenacea*
22. *Drechslera dematioidea*
23. *Exserohilum rostratum*
24. *Exserohilum turcicum*
25. *Acremoniella* spp.
26. *Acremonium* spp.
27. *Alternaria* spp.
28. *Arthrinium* spp.
29. *Aspergillus flavus/A. parasiticus*
30. *Aspergillus niger*
31. *Botrytis* spp.
32. *Cladosporium* spp.
33. *Epicoccum spp.*
34. *Gonatobotrys* spp.
35. *Monilia* spp.
36. *Myrothecium* spp.
37. *Nigrospora* spp.
38. *Papulospora* spp.
39. *Penicillium* spp.
40. *Rhinotrichum* spp.
41. *Stachybotrys* spp.
42. *Stemphylium* spp.
43. *Torula* spp.
44. *Trichoderma* spp.
45. *Trichothecium roseum*
46. *Ulocladium* spp.
47. *Verticillium* spp.
48. *Lasiodiplodia* spp.
49. *Pestalotiopsis* spp.
50. *Phoma* spp.
51. *Septoria nodorum*
52. *Stenocarpella macrospora*
53. *Stenocarpella maydis*
54. *Chaetomium* spp.
55. *Claviceps* spp.
56. *Melanospora* spp.
57. *Sordaria* spp.
58. *Sporisorium reilianum*
59. *Tilletia caries/T. laevis*
60. *Tilletia controversa*
61. *Tilletia indica*
62. *Ustilago maydis*
63. *Ustilago nuda*
64. *Rhizopus* spp.

SEED APPEARANCE

Part or whole seed replaced by spores
55,58,59,60,61,62,63

Seed intact
1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,56,57,64

CHARACTERISTICS OF COLONIES ON SEED

Colony colour

White
10,26,34,40,47,49,50,51,54,56,57,64

White to purple
6,7

White to tan/yellow/orange/brown
2,3,4,5,8,9,11,12,25

White to peach/red
1,3,5,7,9

Cream to orange
35,11

Salmon pink
45,51

Yellow/orange/red
33,38

Blue/green
39

Green to olive brown
27,29,32,36,43,44

Grey
13,17,19,20,21,26,27,28,31,47,51,52,53,57

Brown
14,15,16,17,18,19,20,23,24,27,38,42,56

Black
14,16,20,21,22,26,27,28,30,37,41,42,46,48

Colony type

Compact to cushion-like
2,4,6,12,16,17,19,20,23,26,27,30,32,33,36,37,39,42,43,44,45,46,48,49,51,52,53,54

Loose to spreading
1,3,4,5,6,7,8,9,10,11,12,13,14,15,18,20,21,22,24,25,27,28,29,30,31,32,33,34,35,36,38,39,40,41,42,45,47,48,49,50,51,52,53,54,56,57,64

Development of mycelium

Sparse to moderate
1,3,4,9,11,12,13,15,16,17,19,20,21,22,23,24,25,26,27,30,31,32,33,34,37,38,39,40,41,42,43,44,45,46,47,48,50,52,53,54

Abundant
2,4,5,6,7,8,10,14,18,20,27,28,29,32,33,35,36,45,47,48,49,51,56,57,64

Appearance of spores in mass

Dry
6,8,12,13,14,15,16,17,18,19,20,21,22,23,24,25,27,28,29,30,31,32,33,34,35,37,38,39,40,42,43,44,45,46,54,55,56,57,58,59,60,61,62,63

Wet
1,2,3,4,5,6,7,8,9,10,11,26,36,41,47,48,49,50,51,52,53

Colours of spores in mass

White/Cream
7,8,26,31,34,47,50,51,55

Pink
3,9,11,45

Apricot
35

Orange/red
1,2,3,4,6,10,11,12,38

Green
44

Yellow Green
29

Blue/green
39

Grey
13,31

Brown
4,5,13,14,15,17,18,20,21,22,23,24,25,29,38,48,58,62,64

Purplish brown
28,33

Reddish brown
59,60

Olive brown
19,29,32,43,63

Greenish black
36,41

Black
30,37,42,46,48,49,52,53,54,55,56,57,59,60,61

TAXONOMIC POSITION

Hyphomycetes
1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47

Coelomycetes
48,49,50,51,52,53

Helminthosporium and related genera
13,14,15,16,17,18,19,20,21,22,23,24

Fusarium and Microdochium
1,2,3,4,5,6,7,8,9,10,11,12

Ascomycetes
42,51,54,55,56,57

Ustilaginales (smuts)
58,59,60,61,62,63

Zygomycetes
64

FRUITING STRUCTURES

Spores in relation to fungal structures

Spores formed inside fungal structures
42,48,49,50,51,52,53,54,56,57,64

Spores formed outside fungal structures
1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,55,58,59,60,61,62,63

Type of fruiting structure
Pycnidia
48,50,51,52,53

Sporodochia (cushion-shaped cluster of conidiophores and conidia)
1,2,3,4,5,6,7,8,9,10,11,12,33,36,49

Hyphae
1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,34,35,37,38,39,40,41,42,43,44,45,46,47

Ascocarps
42,51,54,56,57

Sori (mass of spores surrounded by a thin membrane in Ustilaginales)
58,59,60,61,62,63

Sporangia
64

Sclerotia (hard, dark and pigmented resting body)
55

Bulbils (compact irregular clusters of small cells)
38

Shape
Spherical to flask-shaped
42,48,50,51,52,53,54,56,57

Ornamentation
With stiff or wavy hairs (setae)
36,54,56

Without setae
1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,55,57,58,59,60,61,62,63,64

Setae brown
54,56

Setae colourless
36

SPORES

Individual spore colour
Hyaline
1,2,3,4,5,6,7,8,9,10,11,12,26,29,31,34,35,40,44,45,47,50,51,55

Yellow
58,60,64

Yellow-green
29

Olive green
36,44

Blue green
39

Brown
13,14,15,16,17,18,19,20,21,22,23,24,25,27,28,30,32,33,38,41,42,43,46,48,49,51,52,53,54,56,59,61,64

Yellowish brown
63

Olive brown
59,62

Reddish brown
58,59,60

Orange/Red
38

Black
33,37,56,57,58,62

Versicoloured (sections of spore varying in colour)
20,23,49

Cross walls (septa)
Absent
25,26,28,29,30,31,34,35,36,37,39,40,41,44,47,50,54,55,56,57,58,59,60,61,62,63,64

Transverse only
1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,32,43,45,48,49,51,52,53,55

Transverse, longitudinal and oblique
27,33,38,42,46

Number - consistently one
45,48,53

Number - consistently multiseptate
1,2,3,4,5,6,7,8,9,10,13,14,15,16,17,18,19,20,21,22,23,24,49,51,55

Number - variable
11,12,32,43,52

Number - 1-3
1,8,11,12,17,32,31,52

Number - 3-7
1,2,3,4,5,6,7,9,10,13,14,20,21,22,43,49,55

Number - more than 7
15,16,18,19,23,24

Spore with one cell surrounded by outer wall (euseptate)
1,2,3,4,5,6,7,8,9,10,11,12,20,27,32,33,38,42,43,45,46,48,49,51,52,53,55

Spore with individual cells, each surrounded by a sac-like wall distinct from the outer wall (distoseptate)
13,14,15,16,17,18,19,21,22,23,24

Ornamentation
Smooth
1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,31,32,34,35,36,37,38,39,40,41,44,45,47,49,50,51,52,53,54,55,56,57,59

Roughened with tiny warts (verruculose)
27,29,32,39,41,42,43,44,46,61,63

Spiny
30,58,62,63

Roughened with large warts (warty)
33

Marked with lines or grooves or ridges (striate)
36,41,48,64

Polygonal network
59,60

Pitted
59

Mucilaginous (slimy) sheath
57,61

Apical thread-like (filiform) appendages
49

Shape
Crescent-shaped (falcate)
1,2,3,4,5,6,7,8,9,10,11,12,13,15,19

Boat-shaped (fusiform)
1,2,3,4,5,6,7,8,9,10,11,12,13,15,16,18,19,23,24,35,41,49,52,53

Cylindrical
14,17,21,23,24,32,43,51,52,53

Lemon-shaped (limoniform)
54,56,57

Spherical
28,29,30,33,37,38,39,44,58,59,60,61,62,63,64

Lenticular in side view
28

Obovoid (egg-shaped with the apex broader)
22,25,35,40,46

Ovoid (egg-shaped with the base broader)
27,42

Oval (ellipsoid)
26,27,31,34,36,39,41,45,47,48,50,55,64

Slender and thread-like (filiform)
51,55

Unequally curved to one side
20

Size
Width up to 7µm
1,2,3,4,5,6,7,8,9,10,11,12,26,28,29,30,31,32,35,36,39,40,43,44,47,50,51,53,55,64

Width 7-15µm
13,14,17,20,22,31,34,37,41,43,45,48,49,52,54,56,57,58,62,63

Width more than 16µm
15,16,18,19,21,23,24,25,27,33,38,42,46,59,60,61

Length 1-10µm
6,7,8,10,11,26,28,29,30,31,32,35,36,39,40,41,43,44,47,50,54,58,62,63

Length 11-25µm
1,11,12,14,17,25,31,32,33,34,37,42,43,45,46,48,53,56,57,58,59,60,62,64

Length 26-50µm
1,2,3,4,5,6,7,8,9,10,13,14,17,20,21,22,27,38,42,46,49,51,53,61

Length 51-80µm
1,4,6,7,13,16,18,19,21,23,24,27,38,52,55

Length exceeding 80µm
15,16,18,19,21,23,24,27,38,55

Formation
Solitary
1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26

In chains (sometimes breaking up easily)
27,29,30,32,35,39,43,45

Special features
With a foot cell or heel
1,2,3,4,5,6,7,8,9,10,11,12

With a protruding basal scar
23,24

Formed on conspicuous brown conidiophores
13,14,15,16,17,18,19,20,21,22,23,24,27,31,32,42,43,46

Formed on conspicuous pale conidiophores
26,29,30,34,35,39,40,41,44,45,47

MICROCONIDIA

Absent
1,2,3,4,5,9,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64

Present
6,7,8,10,11

CHLAMYDOSPORES

Absent
6,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64

In conidia sometimes
1,2,9

In mycelium
2,3,4,5,7,8,9,10,11,47,50

SCLEROTIA

Absent
1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,30,32,33,34,35,36,37,38,39,40,41,42,43,44,45,46,48,49,50,51,52,53,54,56,57,58,59,60,61,62,63,64

Present
29,31,47,55

BULBILS

Present
38

Fusarium avenaceum (Fr.) Sacc.

Fusisporium avenaceum Fr.

Disease

Head blight or scab, crown rot and foot rot of wheat.

Distribution

Widespread in temperate zones but also found in parts of the humid tropics.

Significance

Crop production: Of lesser virulence and importance than *F. graminearum* and *F. culmorum*, but may cause serious losses as a pre-emergence and seedling blight in cooler climates.

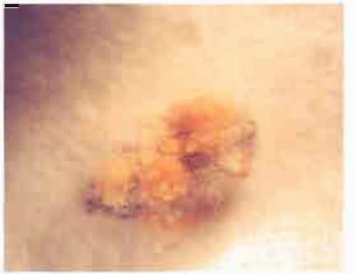
Quarantine: None known.

Detection technique

Freezing blotter method
(See Annex A)

Identification

1



Colony on wheat (x8)

2

Colony on wheat (x32)

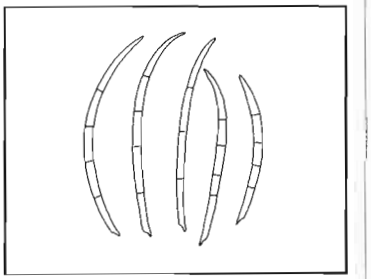
3

Conidiophores and conidia (x400)

4

Conidia (x1000)

Quick clue



References

Booth, C. 1977. Fusarium *Laboratory Guide to the Identification of the Major Species*. CMI, UK.

CMI. 1964. *Descriptions of Pathogenic Fungi and Bacteria No. 25 . Fusarium avenaceum*. CAB, UK.

Nath, R., Neergaard, P., and Mathur, S.B. 1970. Identification of *Fusarium* species on seeds as they occur in blotter test. Proc. Int. Seed Test. Assoc. 35 (1): 121-144.

Zillinsky, F.J. 1983. *Common Diseases of Small Grain Cereals: A Guide to Identification*. CIMMYT, Mexico.

Colony on seed is generally white. Mycelium is white, very fine, cobweb type with tufts and tinged with peach. Blue or violet pigments are absent. Spore masses are bright orange to almost red, formed in big patches on aerial mycelium or sometimes formed in long rows on the seed.

Note: *F. avenaceum* is extremely variable in the appearance of colonies.

Microconidia are absent.

Macroconidia formed from simple conidiophores in the aerial mycelium are hyaline, 1-3 septate, with a conspicuous foot cell, and measure 8-50 x 3-5 µm. Macroconidia formed in clustered conidiophores are hyaline, long, narrow and curve more or less uniformly throughout their length with pointed tips, 4-7 septate, 40-80 x 3-4 µm, and orange in mass.

Chlamydospores absent in mycelium, are rarely present in conidia.

Perithecial state is not confirmed.

Fusarium avenaceum is readily identified by its very long and very narrow, bow-shaped macroconidia with generally more than 3 septa.

The shape of the macroconidia and the absence of both mycelial and conidial chlamydospores separate it from *F. equiseti* and *F. culmorum*. *F. equiseti* is the closest in appearance but has a more prominent foot cell.

Note: *F. avenaceum* is not as widespread as *F. culmorum* and *F. equiseti*.

Fusarium crookwellense Burgess, Nelson & Toussoun

Disease
Stalk rot of maize. Component of "scab" of wheat.

Distribution
Australia, China, Colombia, France, Mexico, South Africa, USA. More abundant in temperate high rainfall or irrigated areas.

Significance
Crop production: As a member of "scab" group of some importance but less than that of *F. graminearum*, *F. culmorum*, or *F. avenaceum*.

Quarantine: None known.

Detection technique
Freezing blotter method
(See Annex A)

Identification

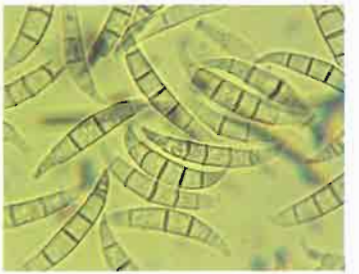
5

Colony on wheat (x8)

6

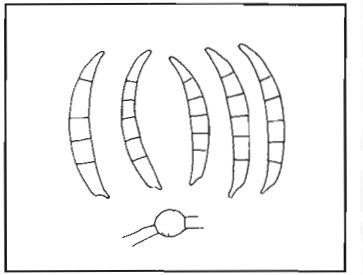
Conidia (x400)

7



Conidia (x1000)

Quick clue



References

Burgess, L.W., Nelson, P.E., and Toussoun, T.A. 1982. Characterization, geographic distribution and ecology of *Fusarium crookwellense* sp. nov. *Trans. Brit. Mycol. Soc.* 79 (3): 497-505.

Nelson, P.E., Toussoun, T.A., and Marasas, W.F.O. 1983. Fusarium *Species - An Illustrated Manual for Identification.* The Pennsylvania State University Press, USA and London.

Colony growth on seed is rapid, with dense aerial mycelium, initially white in colour and then tan. Orange to red-brown spore masses generally appear early in the centre and later in other parts of the colony.

Microconidia are absent.

Macroconidia are hyaline, strongly septate, thick-walled, 3-7 septate and unequally curved, 34-54 x 4-7 µm. The basal cell is distinctly foot-shaped. The apical cell is distinctly curved and tapers to a narrow tip.

Chlamydospores may be present and are formed in the hyphae and the macroconidia.

Macroconidia are readily distinguished by the conspicuous foot at the end of the basal cell, and the distinctly curved apical cell that tapers to a narrow tip.

Macroconidia may be confused with *F. culmorum* or *F. graminearum* or *F. sambucinum*. Macroconidia are longer but not as wide as those from *F. culmorum* or *F. sambucinum*; and shorter and more curved than typical macroconidia of *F. graminearum*. The conspicuous foot at the end of the basal cell is more obvious than that of *F. culmorum* or *F. sambucinum*. The absence of a small projection tip of the apical cell distinguishes *F. crookwellense* from *F. sambucinum*.

Culture on PDA resembles that of *F. culmorum*.

Fusarium culmorum (W.G. Sm.) Sacc.

Fusisporium culmorum W.G. Smith

Disease

Seedling blight, foot and root rot, and head blight of wheat.
Cob and stalk rot of maize.

Distribution

World-wide.

Significance

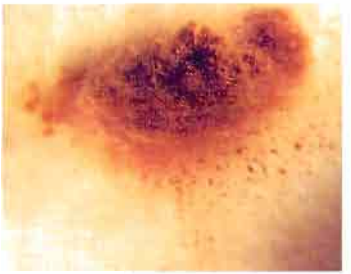
Crop production: Significant yield losses in humid areas. Survives greater extremes of drought and freezing temperatures than *F. graminearum*. Pathogenic to maize seeds.

Quarantine: Restrictions in Brazil.

Note: Associated with mycotoxin (zearalenone) production in maize stalks.

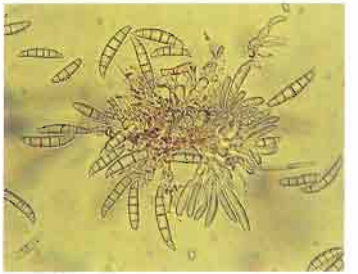
Identification

8



Colony on wheat (x8)

9

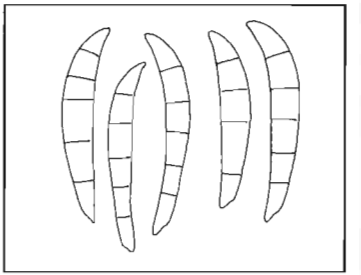


Conidiophores and conidia (x400)

10

Conidiophore and conidia (x1000)

Quick clue



Detection technique

Freezing blotter method
(See Annex A)

References

CMI. 1964. *Descriptions of Pathogenic Fungi and Bacteria No. 26 . Fusarium culmorum*. CAB, UK.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists*. APS Press, USA.

Nath, R., Neergaard, P., and Mathur, S.B. 1970. Identification of *Fusarium* species on seeds as they occur in blotter test. Proc. Int. Seed Test. Assoc. 35 (1): 121-144.

Nelson, P.E., Toussoun, T.A., and Marasas, W.F.O. 1983. Fusarium *Species - An Illustrated Manual for Identification*. The Pennsylvania State University Press, USA and London.

Colony on seed has sparse, very loose mycelium, initially white, then becoming yellow and finally brown or red. There is an abundant production of dull orange to whitish pink spore masses on the seed as well as in the mycelium. These spore masses vary in size and are irregular in shape and slimy in texture.

Microconidia and perithecia are absent.

Macroconidia are formed occasionally on conidiophores produced laterally on aerial mycelium but more frequently they are formed from loosely clustered conidiophores. Macroconidia are short, stout, hyaline, uniformly curved with one side straight and the other curved, with a pointed apical cell and a distinctive foot cell. The conidia are generally 3-5 septate, and measure 26-40 x 4-6 µm.

Chlamydospores are oval to spherical, smooth to rough-walled, single, in chains, or in clumps, and found at intervals along the hyphae.

Distinguished from *F. avenaceum*, *F. graminearum, F. crookwellense*, and *F. sambucinum* by the very uniform, short, distinctly septate, thick-walled, stout macroconidia.

Note: *F. culmorum* is one of the most stable and uniform *Fusarium* sp. although mutants do occasionally occur and these generally show a reduction in pigmentation.

Fusarium equiseti (Corda) Sacc.

Selenosporium equiseti Corda
Fusarium scirpi Lamb. & Fautr.
Teleomorph: *Gibberella intricans* Wollenw.

Disease

Seedling blight and root rot of wheat. Fusarium stalk and root rot of maize.

Distribution

World-wide. Most common in tropical and subtropical areas but also occurs in temperate regions.

Significance

Crop production: Not considered a serious cereal crop pathogen.

Quarantine: None known.

Note: Associated with mycotoxin (zearalenone) production in maize stalks.

Detection technique

Freezing blotter method
(See Annex A)

Identification

11

Colony on wheat (x8)

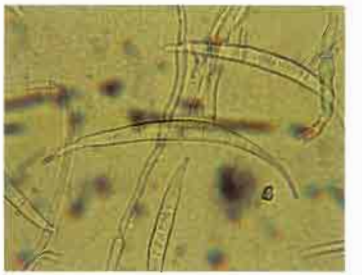
12

Colony on maize (x8)

13

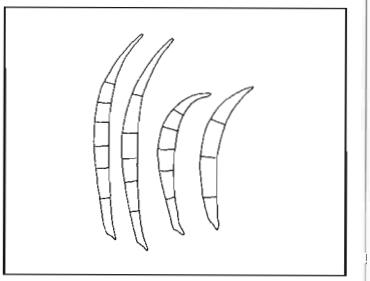
Conidia (x400)

14



Conidia (x1000)

Quick clue



References

CMI. 1978. *Descriptions of Pathogenic Fungi and Bacteria No. 571 . Fusarium equiseti.* CAB, UK.

Nath, R., Neergaard, P., and Mathur, S.B. 1970. Identification of *Fusarium* species on seeds as they occur in blotter test. Proc. Int. Seed Test. Assoc. 35 (1): 121-144.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists.* APS Press, USA.

Zillinsky, F.J. 1983. *Common Diseases of Small Grain Cereals: A Guide to Identification.* CIMMYT, Mexico.

Colony on seed is initially white with white tufted mycelium tinged with peach but later changing to beige and finally deep olive light brownish yellow. Below or within the mycelium light to bright orange or sometimes brown spore masses of different size are present, which are 'dry' in texture. In certain cases very little mycelium is seen on the seed and spore masses arise from the seed surface in long, continuous rows with ridges and furrows from bottom to top. In other cases the mycelium is pale white or light orange, white, fluffy, quite compact, covering the whole seed, and spreading onto the blotter, with no evidence of spore masses when viewed with a stereoscopic microscope. However, in this case orange to brown spore masses can be seen on the seed surface by removing some of the mycelium with a needle.

Microconidia are absent.

Macroconidia are produced from simple or branched conodiophores. Macroconidia are variable in size, hyaline, sickle-shaped, distinctly curved, with a well-developed distinct foot- shaped basal cell and an elongated apical cell which curves inwards. Mature conidia have 4-7 thin but distinct septa and measure 22-60 x 3-6 µm.

Chlamydospores are solitary, found at intervals along hyphae or in chains or knots, spherical, and 7-9 µm diameter with thick roughened walls.

Perithecia are rare and thinly scattered; they are oval with a rough outer wall, and 200-350 µm high x 180-240 µm diameter.

Asci are club-shaped, with 4-8 hyaline, 2-3 septate ascospores which narrow towards the ends and measure 21-33 x 4-6 µm.

Diagnostic characteristics for *F. equiseti* macroconidia are the four to seven distinct septa, a very long elongated and strongly curved (whip-like) apical cell and a well-defined foot cell.

Macroconidia are more or less intermediate in length and width between *F. culmorum* and *F. avenaceum*, and differentiated by the characteristic apical and basal cells.

F. equiseti resembles *F. semitectum* in colony morphology and colour. However, the shape of the macroconidia produced in the aerial mycelium and spore masses are distinctive.

Fusarium graminearum Schwabe

Fusarium roseum Link emend. Snyder & Hansen
Teleomorph: *Gibberella zeae* (Schw.) Petch
Sphaeria zeae Schw.

Disease

Scab, root rot and crown rot of wheat. Seedling blight, stalk and ear rots of maize.

Distribution

World-wide.

Significance

Crop production: Especially important in humid regions. Significant wheat yield losses from floret sterility and poor seed filling. Most destructive of maize stalk rots.

Quarantine: Restrictions for Egypt.

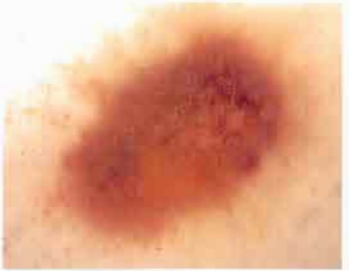
Note: Mycotoxins, formed by pathogen, cause economic losses and reduce germination in maize.

Detection technique

Freezing blotter method
(See Annex A)

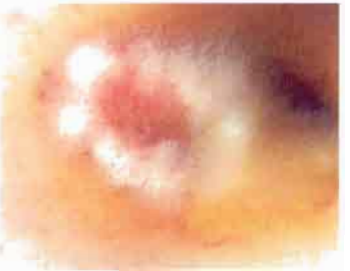
Identification

15



Colony on wheat (x8)

16

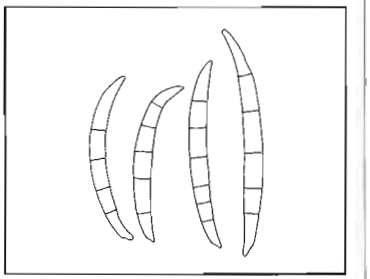


Colony on maize (x8)

17

Conidia (x1000)

Quick clue



References

CMI. 1973. *Descriptions of Pathogenic Fungi and Bacteria No. 384. Gibberella zeae.* CAB, UK.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists.* APS Press, USA.

Sutton, J.C. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum. Can. J. Plant Path.* 4:195-209.

Zillinsky, F.J. 1983. *Common Diseases of Small Grain Cereals: A Guide to Identification.* CIMMYT, Mexico.

Colony on seed with abundant, fine, loose mycelium is initially white, but slowly becomes yellow and finally red. Below the mycelium or within it are present pale to pale brown spore masses of irregular size. Young spore masses are pale white, very slimy and irregular in shape and size.

Note: Infected maize seeds are pink to reddish brown.

Microconidia are absent.

Macroconidia are produced from simple or short multibranched conidiophores; or from clustered conidiophores in older cultures. Conidia are hyaline, straight or slightly curved, 3-7 septate, 25-50 x 3-4 µm, with a well-developed foot-like basal cell, and apical cell curved and sharply pointed.

Chlamydospores if present are found at intervals along hyphae. Chlamydospores are spherical, hyaline to pale brown, single, in chains or clumps; with a smooth or slightly roughened outer wall, and 10-12 µm in diameter.

Oval bluish-black perithecia have a rough outer wall, and measure 140-250 µm diameter.

Asci are club-shaped, with a short foot-stalk (60-85 x 8-11 µm), and contain 8 hyaline to light brown, 1-3 septate, curved, round-ended ascospores, measuring 19-24 x 3-4 µm.

Macroconidia are distinctly long, large, and thick-walled.

Macroconidia are longer and proportionately narrower than those of *F. culmorum.*

In some *F. graminearum* colonies, conidia are not readily produced and identification during seed health tests depends on the recognition of its extremely rapidly growing colonies. Colonies may resemble those of *Fusarium culmorum* and *Fusarium crookwellense* but colonies of these two species become deeply pigmented much more rapidly and normally sporulate freely. The distinction becomes more evident as the colonies grow older. The occasional formation of spore masses and the formation of perithecia help to distinguish *F. graminearum* from *F. culmorum* and *F. crookwellense.*

Fusarium moniliforme J. Sheld.

Lisea fujikuroi Sawada
Fusarium verticillioides (Sacc.) Nirenberg
Teleomorph: *Gibberella fujikuroi* (Sawada) Ito
Gibberella moniliforme Wineland

Disease

Seedling blights of maize and, very rarely, wheat.
Ear and stalk rot of maize.

Distribution

World-wide. Widespread in both humid and subhumid temperate zones and subtropical and tropical zones.

Significance

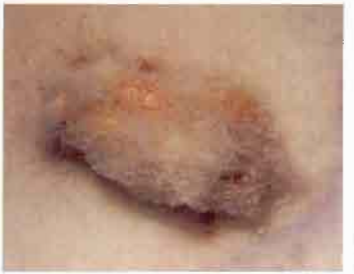
Crop production: The most common maize ear-rot pathogen with significant losses worldwide due to poor crop establishment.

Quarantine: Restrictions for Egypt.

Note: Mycotoxin (moniliformin) formed by pathogen is toxic to humans and livestock when heavily infected grain is consumed.

Identification

18

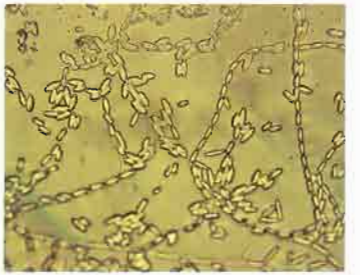


Colony on wheat (x8)

19

Colony on maize (x8)

20

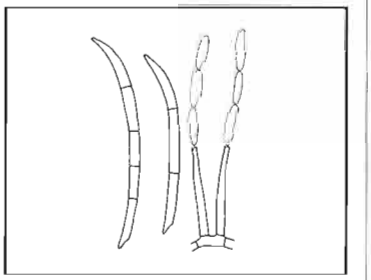


Microconidia (x400)

21

Microconidia and macroconidia (x1000)

Quick clue



Detection technique

Freezing blotter method
(See Annex A)

References

CMI. 1964. Descriptions of Pathogenic Fungi and Bacteria No. 22, *Gibberella fujikuroi.* CAB, UK.

Booth, C. 1971. *The Genus* Fusarium. CMI, UK.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists.* APS Press, USA.

Shurtleff, M.C. 1980. *Compendium of Corn Diseases.* APS Press, USA.

Colony on seed grows rapidly with white aerial mycelium often becoming tinged with purple, particularly on the blotting paper. Mycelium has a powdery appearance due to the presence of chains of microconidia. Tan to orange spore masses of irregular shape and size, are occasionally present.

Note: Infected maize seeds often have white streaks or are rotten.

Microconidia, occurring in abundance, are hyaline, usually one-celled but occasionally two-celled, 5-12 x 1-3 µm, oval to club-shaped and slightly flattened at each end.

Macroconidia occur infrequently, are hyaline, delicate with thin walls and vary from curved to almost straight, 3-7 septate, 25-60 x 2-4 µm, and have a foot-shaped basal cell.

Chlamydospores are never present in mycelium or conidia.

Perithecia, which occur rarely, are spherical, blue-black, and 250-350 µm high by 220-300 µm diameter.

Asci are oval to club-shaped with 4-8 ascospores.

Ascospores are hyaline, straight, mostly one-septate, and measure 4-7 x 12-17 µm.

Abundant uniform microconidia are formed in long chains that can readily be observed using the scotch-tape method (see Annex A) under the microscope at low power (x100).

Chlamydospores are never formed.

Fusarium oxysporum Schlecht.

Disease
None.

Distribution
World-wide.

Significance
Crop production: Negligible; occurring chiefly as a soil saprophyte.

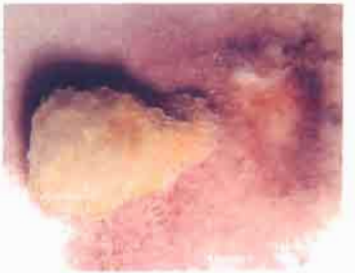
Quarantine: None known.

Note: *Fusarium oxysporum* has been reported to be toxigenic.

Detection technique
Freezing blotter method
(See Annex A)

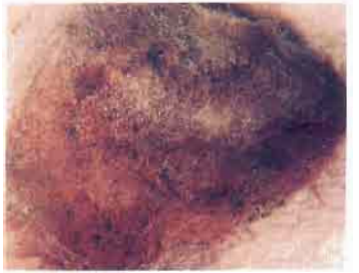
Identification

22



Colony on wheat (x8)

23

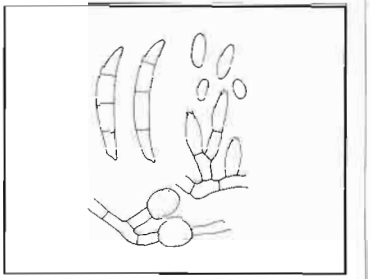


Colony on maize (x8)

24

Conidia (x400)

Quick clue



References

Booth, C. 1971. *The Genus* Fusarium. CAB International, UK.

Booth, C. 1977. Fusarium *Laboratory Guide to the Identification of the Major Species*. CMI, UK.

CMI. 1970. *Descriptions of Pathogenic Fungi and Bacteria No. 211. Fusarium oxysporum*. CAB, UK.

Malone, J.P., and Muskett, A.E. 1964. Seed-borne fungi - description of 77 fungus species. *Proc. Int. Seed Test. Assoc.* 29 (2): 179-384.

Nelson, P.E., Toussoun, T.A., and Marasas, W.F.O. 1983. Fusarium *Species - An Illustrated Manual for Identification*. The Pennsylvania State University Press, USA and London.

Colony on seed is moderately fast growing and produces a varying amount of aerial mycelium, initially white, and changing to peach, salmon, wine grey to purple, violet.

Mycelium becomes thickly matted and sometimes wrinkled in older colonies.

Spore masses are creamy white in colour.

Microconidia borne on short, branched conidiophores, are generally abundant, hyaline, single-celled, variable, oval to kidney shaped, and measure 5-12 x 2-4 µm.

Macroconidia, borne on more elaborately branched conidiophores, are infrequent in some strains.

Macroconidia are hyaline, thin-walled, only slightly curved, pointed at both ends, 3-7 septate, with a somewhat hooked apex and a foot-shaped basal cell; and measure 27-66 x 3-5 µm.

Chlamydospores are spherical, smooth or rough-walled, usually formed singly but occasionally in pairs or in chains, at intervals along hyphae, or on short lateral branches.

Perithecial state is not confirmed.

The presence of chlamydospores, and microconidia borne on short, branched conidiophores are the most distinguishing features of *F. oxysporum*.

The 3 septate macroconidia are most commonly found.

The species may occasionally be confused with *F. moniliforme* if macroconidia and chlamydospores are not readily evident. However, the presence of variable microconidia should help to distinguish *F. oxysporum* from *F. moniliforme*.

Note: *F. oxysporum* is one of the most variable *Fusarium* species.

Fusarium poae (Peck) Wollenw.

Sporotrichum poae Peck
Fusarium poae (Peck) Wollenw. f. *pallens* Wollenw.

Disease

Silver top or white head of wheat. Head blight or white cob-rot of maize.

Distribution

World-wide but predominantly in temperate regions.

Significance

Crop production: Negligible impact.

Quarantine: None known.

Detection technique

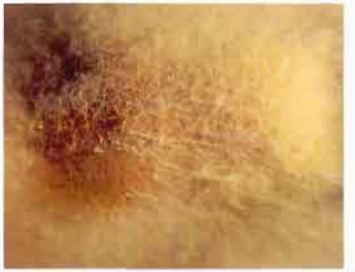
Freezing blotter method
(See Annex A)

Identification

25

Colony on wheat (x8)

26



Colony on maize (x8)

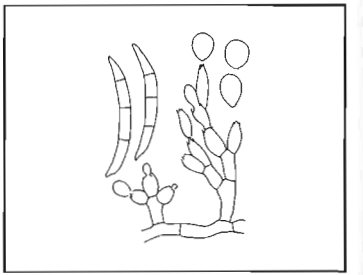
27

Conidiophores and microconidia (x400)

28

Conidiophore and microconidia (x1000)

Quick clue



References

Booth, C. 1971. *The Genus* Fusarium. Kew, England: CAB International.

CMI. 1971. *Descriptions of Pathogenic Fungi and Bacteria No. 308 . Fusarium poae*. CAB, UK.

Malone, J.P., and Muskett, A.E. 1964. Seed-borne fungi - description of 77 fungus species. *Proc. Int. Seed Test. Assoc.* 29 (2): 179-384.

Nath, R., Neergaard, P., and Mathur, S.B. 1970. Identification of *Fusarium* species on seeds as they occur in blotter test. Proc. Int. Seed Test. Assoc. 35 (1): 121-144.

Nelson, P.E., Toussoun, T.A., and Marasas, W.F.O. 1983. Fusarium *Species - An Illustrated Manual for Identification*. The Pennsylvania State University Press, USA and London.

Colony on seed is cottony with fine, white, matted mycelium, and assumes a powdery appearance with the formation of microconidia. Later the aerial mycelium turns reddish-brown.

Conidial masses are generally quite small but variable in size.

Any well-developed colony produces a sweet, very characteristic fruity smell.

Microconidia forming slimy balls, are hyaline, spherical (7-10 µm diameter), or pear-shaped (8-12 x 7-10 µm), and most often 1-celled but occasionally 2-celled (10-14 x 6-7 µm).

Macroconidia are generally rare, hyaline, typically narrowed towards the ends, slightly wider above the middle septum, have a foot-shaped basal cell, and are 3 septate when mature, measuring 20-40 x 3-5 µm.

Chlamydospores occur infrequently and may be in clumps or chains.

The most distinguishing characteristic of *F. poae* is the abundant production of spherical to oval microconidia.

However, it may be easily identified under the stereoscopic microscope, if present as a pure colony on the seed. In well-developed colonies there is abundant loose mycelium and the microconidial masses are irregularly arranged along the hyphae giving the colony a very rough appearance. Such well-developed colonies appear dull white or a little light pink.

Fusarium sambucinum Fuckel

Teleomorph: *Gibberella pulicaris* (Fr.) Sacc.

Disease

Root and seedling rot of wheat.

Distribution

Common in north temperate and Mediterranean regions; Asia, Europe, North Africa and North America.

Significance

Crop production: Relatively minor importance.

Quarantine: None known.

Detection technique

Freezing blotter method
(See Annex A)

Identification

29

Colony on wheat (x8)

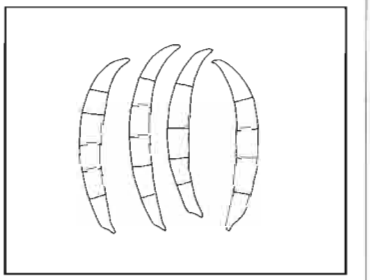
30

Conidia (x400)

31

Conidiophore and conidia (x1000)

Quick clue



References

CMI. 1973. *Descriptions of Pathogenic Fungi and Bacteria No. 385. Gibberella pulicaris.* CAB, UK.

Booth, C. 1971. *The Genus* Fusarium. CMI, UK.

Booth, C. 1977. Fusarium *Laboratory Guide to the Identification of the Major Species.* CMI, UK.

Nelson, P.E., Toussoun, T.A., and Marasas, W.F.O. 1983. Fusarium *Species - An Illustrated Manual for Identification.* The Pennsylvania State University Press, USA and London.

Colony on seed is peach to orange or light brownish yellow, and in some isolates wine-coloured to reddish brown.

Aerial mycelium has white cotton-like groups or tufts tinged with rose.

Microconidia are generally absent.

Macroconidia are produced initially from conidiophores in the aerial mycelium and later from a cushion-shaped cluster of conidiophores. Macroconidia are hyaline, curved, with a pointed apical cell and well-developed foot cell, 3-5 septate, and measure 35-55 x 4-6 µm.

Chlamydospores form infrequently as single spherical cells, 6-11 µm in diameter, either at intervals along hyphae, terminal on short lateral branches or in the cells of the macroconidia, or later form in chains or clumps.

Perithecia are inverted pear-shaped to spherical, and 180-200 µm in diameter. Asci are club-shaped, thin-walled with a slightly thickened apex (70-110 x 11-16 µm) and contain 6-8 spindle-like, curved ascospores with slight constriction at 3 septa and measuring 20-28 x 6-9 µm.

The most distinguishing characteristic of *F. sambucinum* is the shape of the macroconidia with a distinguishing "bird's beak" appearance at the apical cell.

The macroconidia resemble those of *F. culmorum* in length but they are thinner and the constriction and/or curvature of the apical cell is more pronounced.

The shorter length of macroconidia and the presence of a small projection tip of the apical cells distinguish the macroconidia from *F. crookwellense*.

Fusarium tricinctum (Corda) Sacc.

Selenosporium tricinctum Corda
Fusarium citriforme Jamalainen

Disease

Fusarium stalk and root rot of maize.

Distribution

World-wide but more common in temperate regions.

Significance

Crop production: Negligible.

Quarantine: None known.

Note: Pathogen has been reported to be toxigenic.

Detection technique

Freezing blotter method
(See Annex A)

Identification

32

Colony on wheat (x8)

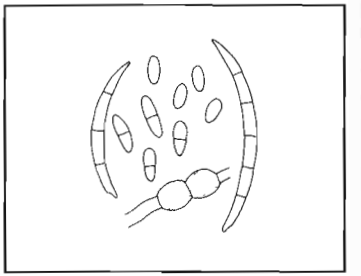
33

Conidiophores and microconidia (x400)

34

Microconidia (x1000)

Quick clue



References

Booth, C. 1971. *The Genus* Fusarium. CMI, UK.

Booth, C. 1977. Fusarium *Laboratory Guide to the Identification of the Major Species.* CMI, UK.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists.* APS Press, USA.

Nelson, P.E., Toussoun, T.A., and Marasas, W.F.O. 1983. Fusarium *Species - An Illustrated Manual for Identification.* The Pennsylvania State University Press, USA and London.

Growth of colony on seed is rapid, and the white aerial mycelium is light to dense, with orange spore masses appearing as the culture ages.

Microconidia are formed initially from simple lateral conidiophores, and later from profusely branched conidiophores. Microconidia are hyaline, abundant, lemon- to pear-shaped or spindle-shaped, 0-1 septate, and often have a foot cell at the base, and measure:
0 septate 7-11 x 4-8 µm
1 septate 10-16 x 4-6 µm.

Macroconidia are abundant, hyaline, usually formed in pale to orange spore masses, sickle-shaped, or more strongly curved, with a well-marked foot cell, 3-5 septate, and measure 26-53 x 3-5 µm.

Spherical chlamydospores (10-12 µm) are present and formed at intervals along hyphae, singly or in chains.

Perithecial state is unknown.

The most distinguishing characteristics are the abundant microconidia that are either lemon- to pear-shaped or broad in the middle and narrowing towards the ends. The shape of the microconidia distinguishes *F. tricinctum* from *F. poae.*

Microdochium dimerum (Penz.) v. Arx

Fusarium dimerum Penz.
Fusarium episphaeria (Tode) Snyder & Hansen
Fusarium aquaeductuum (Radlk. & Rabh.) Lagerh. var. *dimerum* (Penz.) Raillo

Disease

Foot rot of winter wheat.

Distribution

World-wide.

Significance

Crop production: Minor significance.

Quarantine: None known.

Detection technique

Freezing blotter method
(See Annex A)

Identification

35

Colony on wheat (x8)

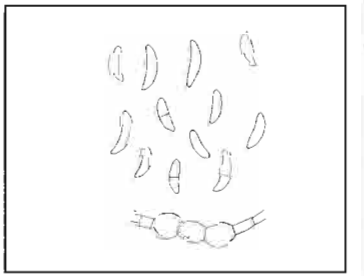
36

Conidia (x400)

37

Conidiophores and conidia (x1000)

Quick clue



References

Booth, C. 1971. *The Genus Fusarium*. CAB, UK.

Booth, C. 1977. Fusarium *Laboratory Guide to the Identification of the Major Species*. CMI, UK.

Nath, R., Neergaard, P., and Mathur, S.B. 1970. Identification of *Fusarium* species on seeds as they occur in blotter test. Proc. Int. Seed Test. Assoc. 35 (1): 121-144.

Nelson, P.E., Toussoun, T.A., and Marasas, W.F.O. 1983. Fusarium *Species - An Illustrated Manual for Identification*. The Pennsylvania State University Press, USA and London.

Colony on seed consists of small, isolated, oval to circular spore masses on the seed surface. They range from dull orange to bright orange or sometimes light pink and are generally smooth. When the infection on the seed is severe, they coalesce to form bigger slimy spore masses covering the entire seed. In most cases there is little mycelium present, and only in the form of a few hyphal strands.

Conidia are crescent shaped, usually non-septate but occasionally have a central septum with the upper cell broader or have two septa. The apical cell may be hooked and the basal cell is blunt or slightly notched. Conidia measure: 0 - septate, 6-11 x 2-3 µm, 1-2 septate, 10-22 x 3-4 µm. Conidia are hyaline when dispersed but salmon pink in mass.

Chlamydospores are spherical or oval, smooth-walled, 8-12 µm in diameter, and formed at intervals along hyphae, singly or in chains.

Sclerotia are also formed.

Perithecial state is unknown.

M. dimerum has the following distinguishing characteristics:

a) very small crescent shaped conidia which are usually non-septate.
b) spore masses coalescing to form bigger slimy conidial masses.
c) colonies with very sparse mycelium.

While the shape of the macroconidia may resemble those of *M. nivale*, macroconidia of *M. dimerum* are smaller. Further, the colony appearance is quite different and *M. dimerum* grows more slowly than *M. nivale* and is capable of producing chlamydospores.

Microdochium nivale (Fr.) Samuels & Hallett

Fusarium nivale Ces. ex Berl. & Vogl.
Teleomorph: *Monographella nivalis* (Schaffn.) E. Müller

Disease

Pre-emergence blight, root rot and occasionally head blight of wheat.

Distribution

Europe, USSR, Japan, Australia, New Zealand, northeastern and northwestern USA, Canada, India, and west Africa.

Significance

Crop production: A serious pathogen of wheat, especially in temperate regions where it may cause total loss of winter sown wheat.

Quarantine: Restrictions in Brazil and other countries.

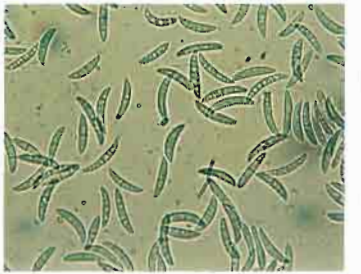
Note: Reported to be toxigenic.

Identification

38

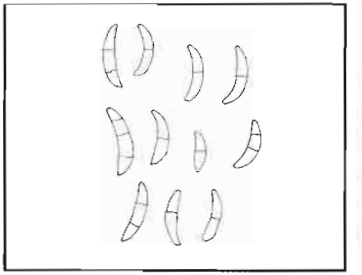
Colony on rice (x8)

39



Conidia (x400)

Quick clue



Detection technique

Freezing blotter method
(See Annex A)

References

CMI. 1971. *Descriptions of Pathogenic Fungi and Bacteria No. 309. Micronectriella nivalis.* CAB, UK.

Nath, R., Neergaard, P., and Mathur, S.B. 1970. Identification of *Fusarium* species on seeds as they occur in blotter test. Proc. Int. Seed Test. Assoc. 35 (1): 121-144.

Wiese, M.V. 1977. *Compendium of Wheat Diseases.* APS Press, USA.

Zillinsky, F.J. 1983. *Common Diseases of Small Grain Cereals: A Guide to Identification.* CIMMYT, Mexico.

Colonies are white to pale peach to apricot with sparse or cotton-like tufts or felty mycelium. Colony on seed has very loose mycelium along with numerous orange spore masses which are irregular in shape and size. The mycelium appears a little pinkish due to the development of spore masses along the hyphae. The spore masses appear circular, smooth and rather 'dry'.

Conidia are hyaline, short, curved, with a pointed apex and flattened, wedge-shaped base, 1-3 septate, but most frequently 1 septate, and measure 10-30 x 2-5 µm.

Chlamydospores are not present.

Perithecia are initially white, but become pink and finally greyish-black. They are oval to spherical, and measure 100-150 x 120-180 µm.

Asci are hyaline, club-shaped, or occasionally cylindrical, thin-walled, 6-9 x 60-70 µm and normally contain 6 to 8 ascospores.

Mature ascospores are hyaline, an oval curve, 2- or 4-celled and measure 3-5 x 10-17 µm.

M. nivale is readily identified by 1-3 septate, short, curved conidia tapering towards the ends, and with foot cells not well-marked.

M. nivale is distinguished from *M. dimerum*, by the following:

a) Generally *M. nivale* has more abundant mycelium than *M. dimerum*.
b) *M. nivale* mycelium is pinkish due to the production of spore masses along the hyphae while *M. dimerum* mycelium is white.
c) Spore masses in *M. nivale* are circular, smooth and rather 'dry', while in *M. dimerum* they are flat, slimy and very irregular in shape.
d) *M. nivale* conidia are longer and always septate.
e) *M. nivale* does not produce chlamydospores.
f) *M. nivale* grows and sporulates best at temperatures of 18ºC or lower.

Bipolaris cynodontis (Marig.) Shoem.

Helminthosporium cynodontis Marignoni
Drechslera cynodontis (Marig.) Subram. & Jain
Teleomorph: *Cochliobolus cynodontis* Nelson

Disease

Leaf blight of maize.

Distribution

World-wide.

Significance

Crop production: Minor importance. Occasional significance only on maize. Causes dark brown lesions, withering and bleaching of leaves.

Quarantine: None known.

Detection technique

Freezing blotter method
(See Annex A)

Identification

40

Colony on wheat (x8)

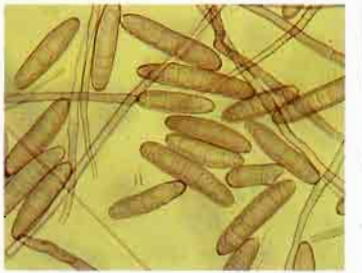
41

Colony on maize (x8)

42

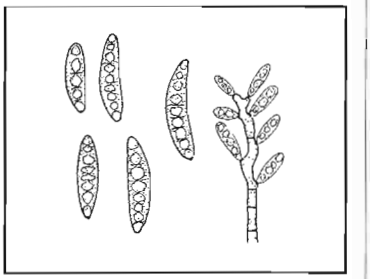
Conidiophores and conidia (x32)

43



Conidia (x400)

Quick clue



References

CMI. 1990. *Descriptions of Pathogenic Fungi and Bacteria No. 1001. Cochliobolus cynodontis.* CABI, UK.

Chidambaram, P., Mathur, S.B., and Neergaard, P. 1973. Identification of seed-borne *Drechslera* species. *Friesia* 10: 165-207.

Nelson, R.R. 1964. The perfect stage of *Helminthosporium cynodontis. Mycologia* 56: 64-69.

Colony on seed grows fairly rapidly and appears grey in colour; with little white aerial mycelium and a large number of conidiophores arising from the seed surface or the blotter.

Conidiophores are single or in small groups, short, straight or slightly bent, pale to mid brown, smooth, cylindrical, septate, up to 170 µm long, and 5-7 µm thick.

Conidia are mostly slightly curved, sometimes almost cylindrical, usually broadest in the middle tapering towards the rounded ends, pale to mid golden brown, smooth, dark scar within the basal cell, 3-9 (commonly 7-8) septate, and measure 30-75 x 10-16 µm.

Pseudothecia are black, spherical or an oval curve, 280-460 x 230-400 µm with an inverted cone shaped beak (30-90 µm long).

Asci are cylindrical to club-shaped, short-stalked, 140-210 x 16-28 µm with 1-8 ascospores.

Ascospores are slender and thread-like, colourless, 3-9 septate, 160-320 x 5-10 µm, and tightly coiled inside the ascus.

Conidia are straight or slightly curved, broader in the middle with rounded ends, dark scar within the basal cell and 3-9 septa.

Note: Germination of conidia is from both ends, but the end cells sometimes swell to form a more or less spherical, thin-walled vesicle from which the germ tubes may originate.

Bipolaris hawaiiensis (M.B. Ellis) Uchida & Aragaki

Helminthosporium hawaiiense Bugnicourt
Drechslera hawaiiensis (Bugnicourt) Subram. & Jain
Teleomorph: *Cochliobolus hawaiiensis* Alcorn

Disease

Leaf spot of maize.

Distribution

World-wide.

Significance

Crop production: Negligible impact. Minor disease detected in a survey in India.

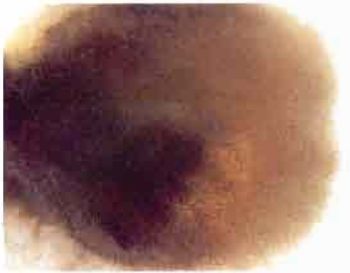
Quarantine: None known.

Detection technique

Freezing blotter method
(See Annex A)

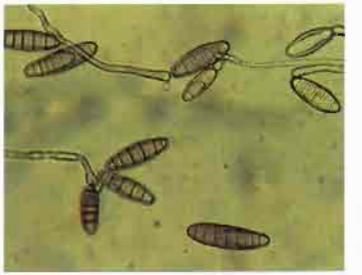
Identification

44



Colony on maize (x8)

45

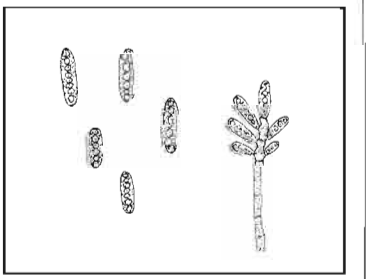


Conidiophores and conidia (x400)

46

Conidia (x400)

Quick clue



References

Chidambaram, P., Mathur, S.B., and Neergaard, P. 1973. Identification of seed-borne *Drechslera* species. *Friesia* 10: 165-207.

CMI. 1982. *Descriptions of Pathogenic Fungi and Bacteria No. 728. Cochliobolus hawaiiensis.* CAB, UK.

Ellis, M.B. 1971. *Dematiaceous Hyphomycetes.* CMI, UK.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists.* APS Press, USA.

Misra, A.P., and Singh, T.B. 1971. Two new leaf spot diseases of maize in India. *Indian Phytopathology* 24:406-407.

Colony on seed spreads loosely, and is black to grey dark brown in colour.

Conidiophores are short, with conidia borne in clusters towards the tip.

Conidiophores are solitary, alternately bent, septate, brown, up to 120 µm long, and 2-7 µm thick.

Conidia are straight, oblong or cylindrical, rounded at the ends, pale to mid brown, 2-7 (mostly 5) septate, and 12-37 x 5-11 µm.

Pseudothecia are spherical, with a long cylindrical neck and 200-450 µm in diameter.

Asci are cylindrical to cylindrically club-shaped, 125-205 x 10-18 µm, and 1-8 spored.

Ascospores are hyaline, slender and thread-like, tapering to a sharp point, loosely coiled in the ascus, 85-190 x 2-6 µm, 4-15 septate, and with a thin hyaline slimy sheath.

Small slender, light to dark, cigar-shaped, cylindric conidia with 4 or more septa are borne in clusters towards the tip of the conidiophore pointing out in different directions.

Growth of colony on seed is similar to *B. spiciferum*, but with shorter conidiophores.

Bipolaris maydis (Nisikado & Miyake) Shoem.

Helminthosporium maydis Nisikado & Miyake
Drechslera maydis (Nisikado & Miyake) Subram. & Jain
Teleomorph: *Cochliobolus heterostrophus* (Drechsler) Drechsler

Disease

Southern leaf blight of maize.

Distribution

World-wide but predominantly in the tropics and subtropics.

Significance

Crop production: Epidemics of disease during 1970 in USA.

Quarantine: Many countries with restrictions including Malaysia. Germplasm with male sterile T cytoplasm is often restricted as well.

Detection technique

Freezing blotter method
(See Annex A)

Identification

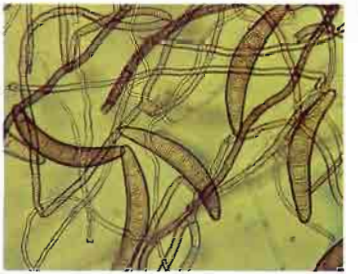
47

Colony on maize (x8)

48

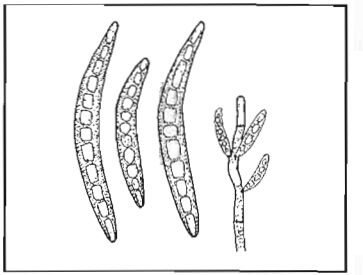
Conidiophore and conidia (x400)

49



Conidia (x400)

Quick clue



References

CMI. 1971. *Descriptions of Pathogenic Fungi and Bacteria No. 301. Cochliobolus heterostrophus.* CAB, UK.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists.* APS, USA.

Singh, D.V., Mathur, S.B., and Neergaard, P. 1974. Seed health testing of maize. Evaluation of testing techniques, with special reference to *Drechslera maydis. Seed Sci. & Technol.* 2: 349-365.

Shurtleff, M.C. 1980. *Compendium of Corn Diseases.* APS Press, USA.

Colony on seed is pale to mid-dark golden brown with some white aerial mycelium, and moderate in density.

Note: A black matted mould may cover the affected maize kernels on the ear and can reduce seed germination.

Conidiophores are mid to dark brown, medium to long, commonly long, slender, straight or curved, single or in groups of 2 or 3, pale near the apex, smooth, up to 700 µm long, and 5-10 µm thick, and bearing conidia at wide intervals.

Conidia are distinctly curved, broad in the middle, sharply tapering towards rounded ends, pale to mid-dark golden brown, smooth, 5-11 septate, mostly 70-160 µm long, 15-20 µm thick in the broadest part; and point of attachment is dark, often flat, and 3-5 µm wide.

Pseudothecia rarely occur under natural conditions; and contain asci with four slender, thread-like, 5-9 septate ascospores (6-7 x 130-340 µm) arranged in parallel coils.

Conidia are light brown, slender, typically curved, and tapering sharply towards both ends. The curvature is more pronounced than in any other related species.

Conidiophores are usually long, slender, alternately bent, and bearing conidia at wide intervals.

Bipolaris sorokiniana (Sacc.) Shoem.

Helminthosporium sativum Pammel, King & Bakke
Drechslera sorokiniana (Sacc.) Subram. & Jain
Helminthosporium californicum Mackie & Paxton
Helminthosporium sorokinianum Sacc.

Helminthosporium acrothecioides Lindfors
Teleomorph:
Cochliobolus sativus (Ito & Kurib.) Drechsler ex Dastur
Ophiobolus sativus Ito & Kurib.

Disease

Black point, seedling blight, common root rot, and spot blotch of temperate cereals.
H. sativum root rot of maize.

Distribution

World-wide.

Significance

Crop production: Major disease of wheat and other temperate cereals. No economic importance on maize.

Quarantine: None known.

Detection technique

Freezing blotter method
(See Annex A)

Identification

50

Colony on wheat (x8)

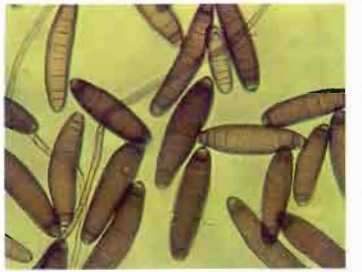
51

Colony on maize (x8)

52

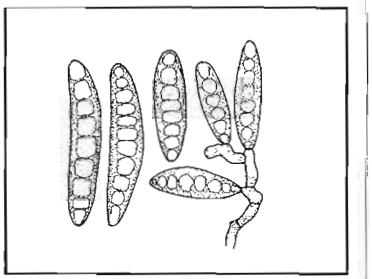
Conidia (x64)

53



Conidia (x400)

Quick clue



References

Chidambaram, P., Mathur, S.B., and Neergaard, P. 1973. Identification of seed-borne *Drechslera* species. *Friesia* 10: 165-207.

CMI. 1981. *Descriptions of Pathogenic Fungi and Bacteria No. 701. Cochliobolus sativus.* CAB, UK.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists.* APS Press, USA.

Zillinsky, F.J. 1983. *Common Diseases of Small Grain Cereals: A Guide to Identification.* CIMMYT, Mexico.

Colony on seed is shiny dark brown to black, composed mainly of dense masses of conidiophores and conidia.

Conidiophores are pale to dark brown, short, erect, in most cases single, solitary or in small groups, straight or alternately bent, up to 220 µm long, 6-10 µm thick and bearing 1-6 conidia at short distances in the upper half.

Conidia are curved to straight, dark olive-brown, smooth, broadest in middle, ends rounded, scar clear within the basal cell, terminal part of end cells subhyaline, 3-12 (mostly 6-10) septate and 40-120 x 17-28 µm.

Pseudothecia are brown to black, flask-shaped, and up to 530 µm broad with a protruding beak 80-110 µm long.

Asci are cylindrical to club-shaped, 1-8 spored, and measure 110-230 x 30-45 µm.

Ascospores are slender and thread-like, hyaline to light brown, 6-13 septate, and measure 160-360 x 6-9 µm.

Conidia appear black and shiny under low magnification, but under higher magnification they are dark olive brown. Conidia are large, thick-walled, typically have five to nine cells, may be straight or slightly curved, and are characteristically somewhat barrel-shaped.

Bipolaris spicifera (Bainier) Subram.

Helminthosporium spiciferum (Bainier) Nicot
Helminthosporium tetramera McKinney
Curvularia spicifera (Bainier) Boedijn
Teleomorph: *Cochliobolus spicifer* Nelson

Disease

Foot rot in winter wheat.
Leaf spot of maize.
Leaf blotch of wheat and barley.

Distribution

World-wide and very common in tropical or subtropical areas.

Significance

Crop production: Impact considered negligible.

Quarantine: None known.

Detection technique

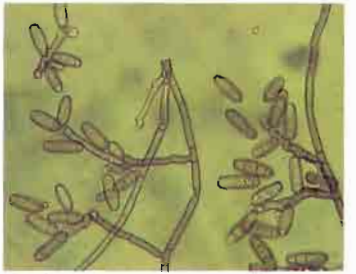
Freezing blotter method
(See Annex A)

Identification

54

Colony on wheat (x8)

55

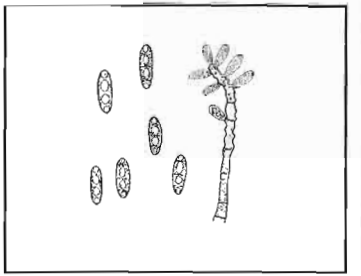


Conidiophores and conidia (x400)

56

Conidia (x400)

Quick clue



References

McGee, D.G. 1988. Maize Diseases: A Reference Source for Seed Technologists. APS Press, USA.

Ellis, M.B. 1971. *Dematiaceous Hyphomycetes*. CMI, UK.

Zillinsky, F.J. 1983. *Common Diseases of Small Grain Cereals*. CIMMYT, Mexico, D.F.

Colony on seed is brown, grey or black, hairy, cottony or cushion-like and spreads loosely with abundant brownish conidiophores, single or in clusters of 2 to 3. Many small conidia are produced at very short intervals, giving rise to a bottle-brush appearance.

Colonies strongly resemble those of *Curvularia* spp.

Conidiophores are brown, and curved, with obvious and numerous scars resulting in an irregular zig-zag appearance.

Conidia are short, typically 3-septate, light to dark-brown, oval, curved to straight with rounded ends, and measure 20-40 µm x 9-14 µm. Conidia are lighter in colour towards the terminal cells.

Ascomata are black, spherical to oval curve, 460-710 x 350-650 µm, with an inverted cone shaped neck and pore-opening.

Asci are cylindrical to club-shaped, straight to slightly curved, 1-8 spored and measure 130-160 x 12-20 µm.

Ascospores are parallel to closely coiled in the ascus, thread-like, somewhat tapered at the ends, 6-16 septate, hyaline, and measure 135-240 x 3-7 µm.

Under the dissecting microscope, conidia appear to be clustered for some length on the conidiophores, giving the appearance of a bottle brush.

Conidia are very small and typically 3-septate, almost cylindrical, more or less uniform in size, and the end cells have sub-hyaline areas towards their terminal ends.

Bipolaris victoriae (Meehan & Murphy) Shoem.

Helminthosporium victoriae Meehan & Murphy
Drechslera victoriae (Meehan & Murphy) Subram. & Jain
Teleomorph: *Cochliobolus victoriae* Nelson

Disease

Leaf spot of wheat.
Victoria blight of oats.

Distribution

Australia, Europe, North America.

Significance

Crop production: Negligible on wheat. None on maize.

Quarantine: Restrictions by IAPSC (Africa) on list A1 (not present in region).

Detection technique

Freezing blotter method
(See Annex A)

Identification

57

Colony on wheat (x8)

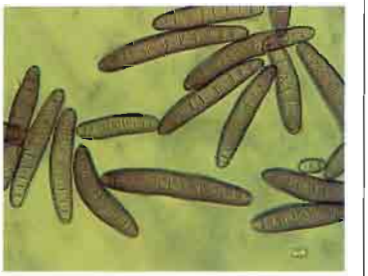
58

Colony on blotter (x64)

59

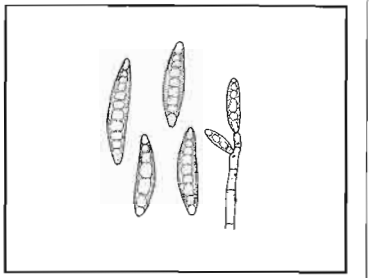
Conidia (x160)

60



Conidia (x400)

Quick clue



References

Chidambaram, P., Mathur, S.B., and Neergaard, P. 1973. Identification of seed-borne *Drechslera* species. *Friesia* 10: 165-207.

Ellis, M.B. 1971. *Dematiaceous Hyphomycetes*. CMI, UK.

Colony on seed is shiny dark brown, and composed mainly of dense masses of conidiophores and conidia. Growth is fairly rapid, and spreads onto surrounding blotter paper.

Conidiophores are solitary or in groups, straight or alternately bent, pale to mid-brown, up to 250 µm long, and 6-10 µm thick.

Conidia are slender, slightly curved, narrowed towards the ends or club-shaped, light golden brown, smooth, 4-11 septate, and measure 40-120 x 12-19 µm.

Ascomata are spherical to an oval curve, 225-430 x 210-370 µm, with stiff hairs on the upper half, and an inverted cone-shaped neck 30-170 µm long with pore opening.

Asci are cylindrical to club shaped, with short stalk, two walls, 1-8 spores, and measure 98-207 x 20-39 µm.

Ascospores are thread-like, hyaline, slightly tapered at the ends, 5-9 septate, coiled in the ascus, and measure 147-302 x 6-13 µm.

The growth characteristics on seed are very similar to those of *H. sativum* except the conidia of *B. victoriae* are a little lighter in colour, slender and slightly curved.

Bipolaris zeicola (Stout) Shoem.

Helminthosporium carbonum Ullstrup
Drechslera zeicola (Stout) Subram. & Jain
Teleomorph: *Cochliobolus carbonum* Nelson

Disease

Black ear rot; carbonum leaf spot; northern leaf spot; and Helminthosporium leaf spot of maize.

Distribution

World-wide.

Significance

Crop production: Minor disease although commonly found on stalks and leaves. Ear rots are only found occasionally. Moderate temperatures and high relative humidity favour the disease.

Quarantine: Restrictions for Indonesia, Egypt, Chile, and by NEPPO (Near East) on list A1 (not present in region); and by EPPO (Europe) and IAPSC (Africa) on list A2 (present in part of the region, of quarantine importance elsewhere).

Identification

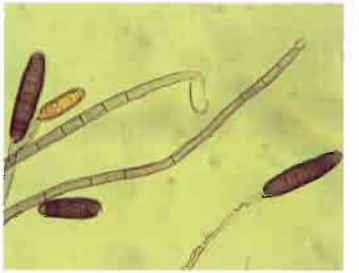
61

Colony on maize (x8)

62

Colony on maize (x64)

63

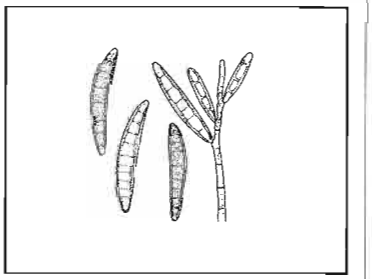


Conidiophores and conidia (x400)

64

Conidia (x400)

Quick clue



Detection technique

Freezing blotter method
(See Annex A)

References

CMI. 1972. *Descriptions of Pathogenic Fungi and Bacteria No. 349. Cochliobolus carbonum.* CAB, UK.

Ellis, M.B. 1971. *Dematiaceous Hyphomycetes.* CMI, UK.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists.* APS Press, USA.

Shurtleff, M.C. 1980. *Compendium of Corn Diseases.* APS Press, USA.

Colony on seed is often deep-seated and grey-brown, with denser matted colonies greyish. Mycelium has dark thick-walled hyphae.

Note: Infected seeds on a maize ear are covered with very dark brown or black mycelium giving them a characteristic charcoal appearance.

Conidiophores arise singly or in small groups, are straight or curved, mid to dark brown or olive brown, up to 250 µm long, and 5-8 µm thick.

Conidia are curved or sometimes straight, occasionally almost cylindrical but usually broader in the middle and tapering towards the rounded ends, 30-100 x 12-18 µm (mostly 60-80 x 14-16 µm), 6-12 (usually 7-8) septate, golden yellow to very dark olive-brown, end cells sometimes paler than the middle ones; point of attachment is not very conspicuous.

Pseudothecia are spherical and black, containing cylindrical or club-shaped, straight or slightly curved asci.

Ascospores are slender, thread-like, hyaline, 5-9 septate, 6-10 x 182-300 µm and coiled in the ascus.

Diagnostic features are dark thick-walled hyphae and yellow to very dark olive-brown, straight or curved conidia with 6-12 (usually 7-8) septa.

Curvularia Boedijn

Disease

Curvularia leaf spot of wheat and maize (*C. lunata; C. pallescens; C. inequalis; C. tuberculata; C. maculans; C. clavata*).

Distribution

World-wide - especially in the tropics.

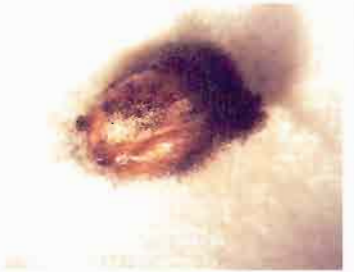
Significance

Crop production: Frequently encountered as a pathogen or saprophyte of maize and wheat. *C. pallescens* causes a late season disease of maize that can result in serious losses in tropical regions, but is a minor pathogen in temperate regions.

Quarantine: None known.

Identification

65

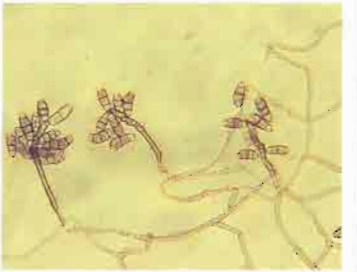


Colony on wheat (x8)

66

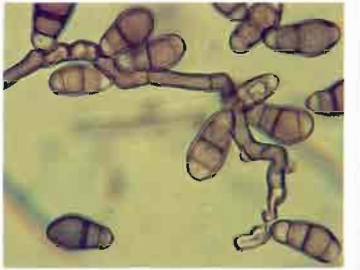
Colony on maize (x8)

67



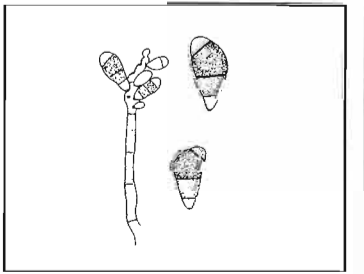
Conidiophores and conidia (x400)

68



Conidiophore and conidia (x1000)

Quick clue



Detection technique

Freezing blotter method
(See Annex A)

Colony on seed is brown, grey or black, hairy, cottony or cushion-like, and spreads loosely.

Hyphae are branched, septate, colourless or brown, smooth or with rough swellings. Stromata are often large, erect, black, cylindrical, and sometimes branched.

Conidiophores arise singly or in groups, terminally and laterally on the hyphae, also on stromata when these are present; and are brown, septate, straight or curved, simple or branched.

Conidia are borne at the apex or sides of the conidiophore. Conidia are straight or curved, usually broad in the middle and narrow towards the ends, an oval, an inverted egg shape, club-shaped or pear-shaped, occasionally rounded at the base, or with a distinct point of attachment, 3 or more septate, smooth, or rough, and often with one or more middle cells larger and darker than the others.

The dark, slightly curved conidia with broader middle cells and paler end cells, are typical of the common species of *Curvularia*.

Species may be confused superficially with small-spored species of *Drechslera* and *Bipolaris*.

References

Barron, G.L. 1972. *The Genera of Hyphomycetes from Soil.* R.E. Kreiger Publishing Co., USA.

Benoit, M.A., and Mathur, S.B. 1970. Identification of species of *Curvularia* on rice seed. *Proc. Int. Seed Test. Assoc.* 35 (1): 99-119.

Ellis, M.B. 1966. Dematiaceous hyphomycetes. VII. *Curvularia, Brachysporium*, etc. *CMI Mycological Papers*, No. 106.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists.* APS Press, USA.

Drechslera avenacea (Curtis ex Cooke) Shoem.

Helminthosporium avenae Eidam
Helminthosporium avenaceum Curtis ex Cooke
Teleomorph: *Pyrenophora chaetomioides* Speg.

Disease

Helminthosporium blight of oats.

Distribution

World-wide.

Significance

Crop production: No significance on wheat or maize.

Quarantine: None known.

Detection technique

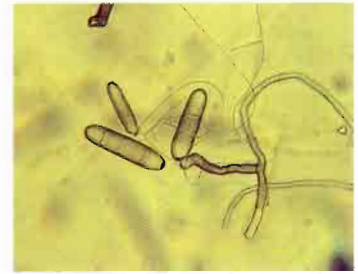
Freezing blotter method
(See Annex A)

Identification

69

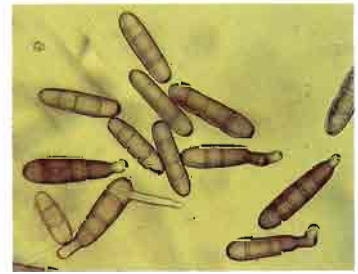
Colony on wheat (x10)

70



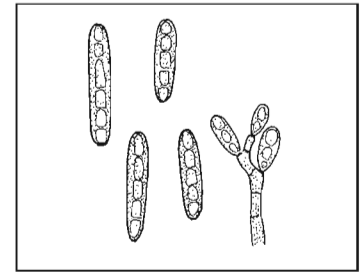
Conidiophore and conidia (x400)

71



Conidia (x400)

Quick clue



References

Chidambaram, P., Mathur, S.B., and Neergaard, P. 1973. Identification of seed-borne *Drechslera* species. *Friesia* 10: 165-207.

Ellis, M.B. 1971. Dematiaceous Hyphomycetes. CMI, UK.

Shoemaker, R.A. 1962. *Drechslera* Ito. *Canadian Journal of Botany* 40: 809-836.

Colony on seed is blackish grey, with or without greyish white to white tufts, with little aerial mycelium and largely conidiophores bearing conidia.

Conidiophores are single or in groups of 2 to 3, straight to alternately bent, infrequently branched, dark reddish brown, and up to 1mm long.

Conidia are mostly cylindrical with semi-circular rounded ends, light green to medium yellowish brown, lighter coloured basal cell, very conspicuous, nearly black, 4-9 µm wide scar and average 25-140 x 12-22 µm with 2-6 septa.

Ascomata are spherical to sub-spherical, with stiff dark brown hairs and measure 450-800 x 300-600 µm with a short cylindrical beak. Asci are cylindrical to club-shaped, 2-8 spored, straight to slightly curved, with short stalk, and measure 180-400 x 32-60 µm.

Ascospores are hyaline to light yellow brown, oval to cylindrical, rounded at both ends, with 3-6 transverse septa and 0-1 longitudinal septa in all cells and measure 35-75 x 17-30 µm.

Conidia are cylindrical with straight sides and semi-circular rounded ends, 2-6 septate and a conspicuous scar.

Drechslera dematioidea (Bub. & Wrób.) Subram. & Jain

Helminthosporium dematioideum Bub. & Wrób.

Disease

None.

Distribution

World-wide.

Significance

Crop production: Negligible impact; associated with inflorescence as well as withered or dead leaves of several grass species.

Quarantine: None known.

Detection technique

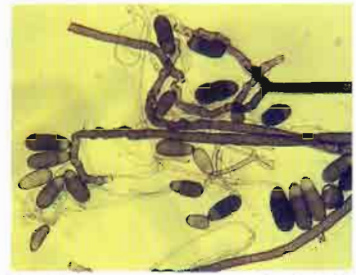
Freezing blotter method
(See Annex A)

Identification

72

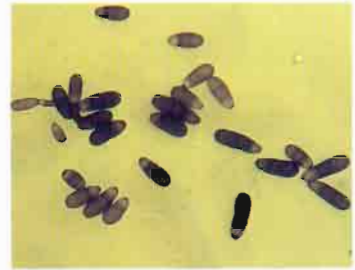
Colony on wheat (x8)

73



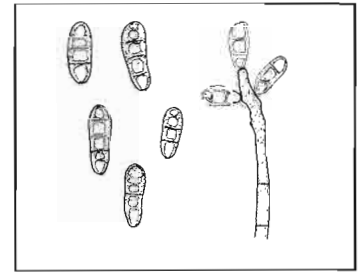
Conidiophores and conidia (x400)

74



Conidia (x400)

Quick clue



References

Chidambaram, P., Mathur, S.B., and Neergaard, P. 1973. Identification of seed-borne *Drechslera* species. *Friesia* 10: 165-207.

Colony on seed has a black appearance with sparse to moderate growth of mycelium.

Conidiophores are light brown, short, slender, and arise singly or in pairs.

Conidia are yellowish brown, with the basal cell lighter in colour, club-shaped, broader at the tip, tapering towards the base and ending in a wide dark scar. Conidia average 24-40 x 9-15 µm with 3-5 septa.

Conidia are 3-5 septate, club-shaped, broader at the tip, tapering towards the base with the basal cell lighter in colour and a wide dark basal scar.

Conidia are formed on light brown conidiophores arising singly or in pairs.

Exserohilum rostratum (Drechsler) Leonard & Suggs

Helminthosporium rostratum Drechsler
Drechslera rostrata (Drechsler) Richardson & Fraser
Bipolaris rostrata (Drechsler) Shoemaker
Teleomorph: *Setosphaeria rostrata* Leonard

Disease

Leaf spot and foot rot of wheat.
Stalk rot and ear rot of maize.

Distribution

Africa, Asia, Central America, Europe, North America.

Significance

Crop production: Minor disease of limited concern.

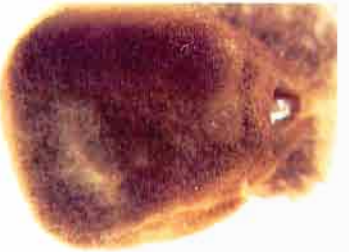
Quarantine: None known.

Detection technique

Freezing blotter method
(See Annex A)

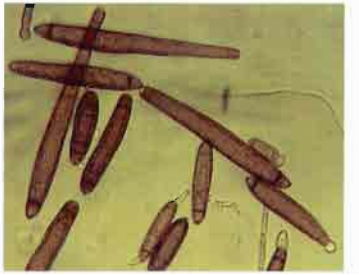
Identification

75



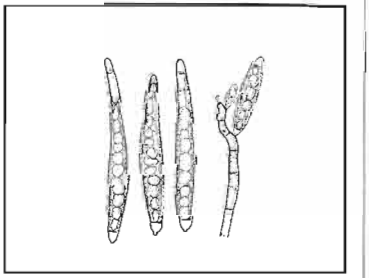
Colony on maize (x8)

76



Conidia (x400)

Quick clue



References

CMI. 1978. *Descriptions of Pathogenic Fungi and Bacteria No. 587. Setosphaeria rostrata.* CAB, UK.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists.* APS Press, USA.

Kucharek, T.A. 1973. Stalk rot of corn caused by *Helminthosporium rostratum*. *Phytopathology* 63 (11): 1336-1338.

Whitehead, M.D., & Calvert, O.H. 1959. *Helminthosporium rostratum* inciting ear rot of corn and leaf spot of thirteen grass hosts. *Phytopathology* 49: 817-820.

Colony on seed appears mid to dark brown or golden brown with very little white aerial mycelium.

Conidiophores are formed together in a dense mat covering the seed.

Note: Infected maize kernels show pink discolouration or are charcoal black when severely infected.

Conidiophores are solitary or in groups, straight or bending, mid to dark brown or olive-brown, up to 200 µm long and 8 µm thick.

Conidia are straight or slightly curved, tapering to both ends with one end typically wider, and the narrow end terminating in a pronounced beak. Conidia have 6-16 transverse septa, hyaline or pale end cells with a thick dark septum, golden brown intermediate cells; and measure 40-180 x 14-22 µm.

Ascocarps are spherical, black, 340-600 x 330-580 µm, with pore opening and upper part surrounded with dark brown, blunt spine-like projections.

Asci are short-stalked, club-shaped to cylindrical, 1-8 spored, and measure 105-260 x 26-42 µm. Ascospores are hyaline to pale brown, straight to curved, 2-5 septate, narrowed at septa, 29-85 x 9-21 µm, with a slimy sheath.

Conidia have a distinctive shape and are straight or slightly curved, with a pronounced beak, and visible dark end septa.

Exserohilum turcicum (Pass.) Leonard & Suggs

Helminthosporium turcicum Pass.
Drechslera turcica (Pass.) Subram. & Jain
Helminthosporium inconspicuum Cooke & Ellis
Teleomorph: *Setosphaeria turcica* (Luttrell) Leonard & Suggs

Disease

Northern leaf blight of maize.

Distribution

World-wide but predominantly in subtropical to temperate climates.

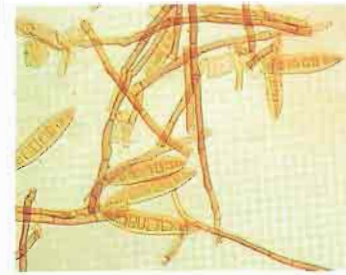
Significance

Crop production: Disease commonly occurs, but usually only causes minor losses. Severe outbreaks occasionally occur with susceptible varieties under cool temperatures, and heavy and frequent dews.

Quarantine: Restrictions for some countries.

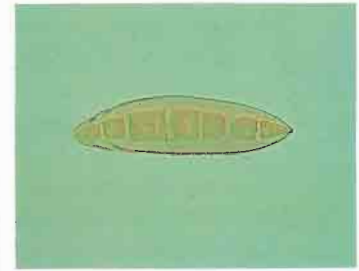
Identification

77



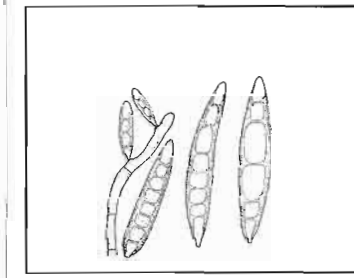
Conidiophores and conidia (x400)

78



Conidia (x1000)

Quick clue



Detection technique

Freezing blotter method
(See Annex A)

Colony on seed is pale to mid-dark brown with very little white aerial mycelium.

Conidiophores are single or in groups of 2-6, straight or bent, light to dark olive-brown, medium to long, sometimes very long, and measure 150-300 x 7-11 µm.

Conidia are straight or slightly curved, club-shaped or widest near the middle, tapering towards the ends, with a rounded apex, and basal cell bulging out at the point of attachment. Conidia are pale to mid straw coloured or yellowish brown or olive grey in colour, 4-9 septate, and measure 50-144 x 18-33 µm.

Perithecia are black and spherical.

Asci are cylindrical with a short stalk, and contain 1-8 (usually 2-4) ascospores which are hyaline, straight or slightly curved, typically 3-septate, and measure 13-17 x 42-78 µm.

Note: Perithecia rarely occur in nature.

Conidia arise from long conidiophores and are large, yellowish brown, straight, or slightly curved, narrowing towards both ends (almost cigar shaped), with the basal cell bulging out at the point of attachment.

References

Chidambaram, P., Mathur, S.B., and Neergaard, P. 1973. Identification of seed-borne *Drechslera* species. *Friesia* 10: 165-207.

CMI. 1971. *Descriptions of Pathogenic Fungi and Bacteria No. 304. Trichometasphaeria turcica.* CAB, UK.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists.* APS Press, USA.

Shurtleff, M.C. 1980. *Compendium of Corn Diseases.* APS Press, USA.

Acremoniella Sacc.

Disease
None.

Distribution
World-wide.

Significance
Crop production: Common saprophyte and secondary invader.

Quarantine: None known.

Detection technique
Freezing blotter method
(See Annex A)

Identification

79

Colony on wheat (x8)

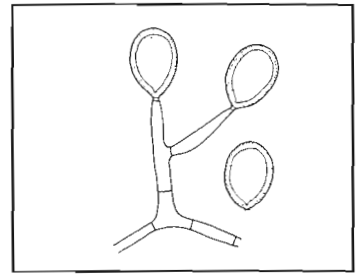
80

Colony on blotter (x64)

81

Conidiophores and conidia (x400)

Quick clue



References

Barron, G.L. 1972. *The Genera of Hyphomycetes from Soil*. R.E. Kreiger Publishing Co., New York, USA.

Malone, J.P., and Muskett, A.E. 1964. Seed-borne fungi - description of 77 fungus species. *Proc. Int. Seed Test. Assoc.* 29 (2): 179-384.

Colony on seed grows rapidly producing a thin cobwebby mycelium with numerous grape-like clusters of brown conidia.

Note: Species grow more vigorously when in association with certain other fungi such as *Alternaria tenuis*, *Fusarium moniliforme*, and *Acremonium strictum*.

Conidiophores are hyaline, septate, and either simple or branched several times (often at right angles). They are usually more than 10 µm long, 4-7 µm wide, and sharply tapering towards the tip.

Conidia are large, brown, solitary, continuous, egg shaped, smooth (*A. atra*) or rough (*A. verrucosa*), light brown in colour, and measure 22-29 x 18-22 µm.

Little mycelium with clusters of large, brown, egg shaped conidia borne singly on sharply pointed conidiophores.

Acremonium strictum

Cephalosporium acremonium Corda
Acremonium kiliense Grütz
Cephalosporium madurae Padhye, Sukapure & Thirumalachar

Disease

Black bundle disease of maize.

Distribution

World-wide - more frequent in the tropics.

Significance

Crop production: Black bundle disease of maize is a minor, late season disease of common occurrence in USA and other countries.

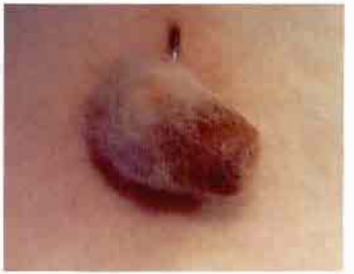
Quarantine: None known.

Detection technique

Freezing blotter method
(See Annex A)

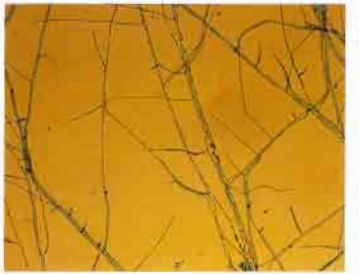
Identification

82



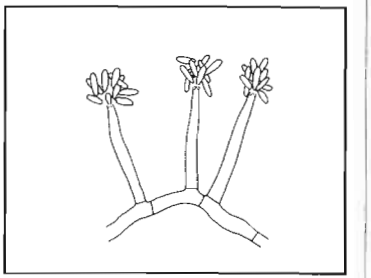
Colony on wheat (x8)

83



Conidiophores and conidia (x400)

Quick clue



References

Barron, G.L. 1972. *The Genera of Hyphomycetes from Soil.* Robert E. Kreiger Publishing Co., USA.

CMI. 1983. *Descriptions of Pathogenic Fungi and bacteria No. 741. Acremonium kiliense.* CAB, UK.

Gams, W. 1971. *Cephalosporium - artige Schimmelpilze (Hyphomycetes).* Gustav Fischer Verlag, Stuttgart, Germany.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists.* APS Press, USA.

Zillinsky, F.J. 1983. *Common Diseases of Small Grain Cereals: A Guide to Identification.* CIMMYT, Mexico.

Colony on seed is compact, slow growing, white to pale grey and becoming slate grey or black with age. Hyphae are hyaline, septate, simple or branched. The hyphae are often grouped together forming threads and along the sides of the threads numerous solitary conidiophores are formed, each with its globule of spores.

Note: Infected maize seeds can show white streaks on the seed surface.

Conidiophores arising directly and singly at right-angles from the vegetative hyphae, are hyaline, short, tapered towards the tip; and measure 30-60 µm long and 1.5 µm at the base.

Conidia are borne in a slimy matrix in balls or rarely in fragile chains at the apex of the conidiophore. Conidia are hyaline, cylindrical with rounded ends, sometimes curved, non-septate, and measure 3-10 x 1-2 µm.

The key characteristic of *Acremonium* is the ball of spores produced on top of solitary, tapering conidiophores, usually borne at right angles to the hyphae.

Note: The genus can be readily confused with others such as *Gliomastix*, *Verticillium*, and microconidial *Fusarium* or *Cylindrocarpon*. Nevertheless, it is perhaps one of the easiest fungi to identify to genus and one of the most difficult in which to make species determinations.

Alternaria Nees

Disease

Alternaria leaf blight of wheat (*A. triticina*) and maize (*A. alternata*); and grey leaf spot of maize (*A. maydis*).

Distribution

World-wide.

Significance

Crop production: Most species exist as saprophytes, but some, such as *A. triticina*, cause severe blight on leaves and spikes of wheat and triticale, but do not infect barley or oats. *A. alternata* (*A. tenuis*) is of minor importance on maize.

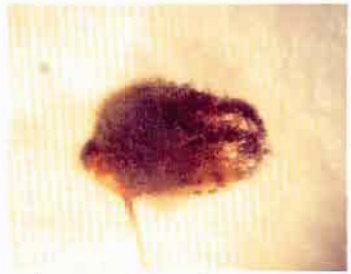
Quarantine: *Alternaria triticina* is on many quarantine lists.

Detection technique

Freezing blotter method

Identification

84

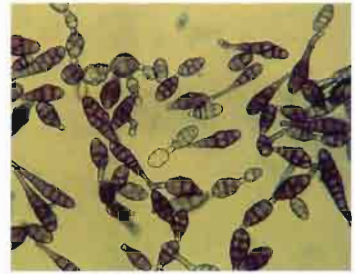


Colony on wheat (x8)

85

Colony on maize (x8)

86

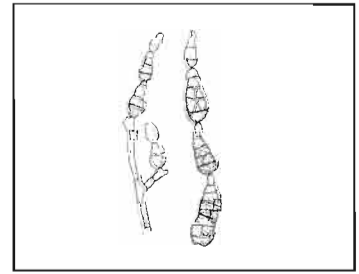


Conidia (x400)

87

Conidiophore and conidia (x1000)

Quick clue



References

Barron, G.L. 1972. *The Genera of Hyphomycetes from Soil.* Robert E. Kreiger Publishing Co., USA.

Ellis, M.B. 1971. *Dematiaceous Hyphomycetes.* CMI, UK.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists.* APS Press, USA.

Simmons, E.G. 1967. Typification of Alternaria, Stemphylium and Ulocladium. *Mycologia* 59:67-92.

Shurtleff, M.C. 1980. *Compendium of Corn Diseases.* APS Press, USA.

Zillinsky, F.J. 1983. *Common Diseases of Small Grain Cereals: A Guide to Identification.* CIMMYT, Mexico.

Colony on seed is usually dark grey in colour, but may also be white, olive green, brown, or almost black.

Conidiophores are dark to olive brown, arise singly or occasionally in small groups, may be simple or branched and usually measure 3-6 µm thick and 50 µm long.

Conidia of most of the saprophytic *Alternaria* species that occur on cereals develop in chains, are egg-shaped or an oval, and often taper to a beak at the apex. They are medium to dark brown in colour, with smooth or slightly roughened walls, with several transverse and longitudinal or oblique septa, and measure 20-90 x 8-20 µm.

The species that cause leaf spots develop singly or in chains, have somewhat larger conidia, up to 100 µm long and 30 µm wide, but are very similar in other characteristics.

The distinctive pale brown, beaked, conidia with transverse and longitudinal septa are readily identifiable in most cases.

Note: Conidia of *Alternaria* species have unique morphological characteristics by which this genus can be readily identified. However, similarities among species within the genus and the variability in shape, size and septation of conidia within a species make species identification very difficult.

Arthrinium Kunze

Papularia Fr.

Disease

None.

Distribution

World-wide.

Significance

Crop production: None. Common saprophyte and secondary invader.

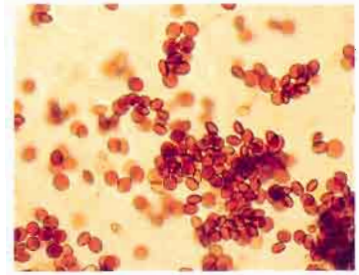
Quarantine: None known.

Detection technique

Freezing blotter method (See Annex A)

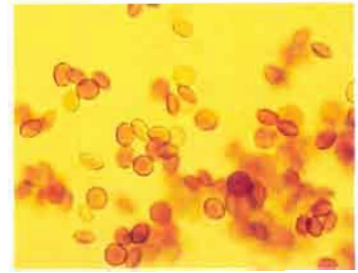
Identification

88



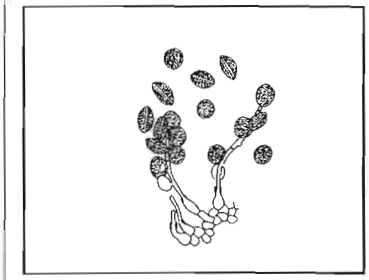
Conidia (x160)

89



Conidia (x400)

Quick clue



References

Barron, G.L. 1972. *The Genera of Hyphomycetes from Soil*. R.E. Kreiger Publishing Co., New York, USA.

Ellis, M.B. 1971. *Dematiaceous Hyphomycetes*. CMI, UK.

IMI. 1991. *Descriptions of Pathogenic Fungi and Bacteria No. 1052, Apiospora montagnei*. CABI, UK.

Malone, J.P., and Muskett, A.E. 1964. Seed-borne fungi - description of 77 fungus species. *Proc. Int. Seed Test. Assoc.* 29 (2): 179-384.

Colony on seed initially has abundant white aerial mycelium, and becomes grey to black in colour with the production of conidia in large masses.

Conidiophores are hyaline except for the thick transverse septa which are often purplish brown to dark brown.

Conidia are often formed in tight clusters along and at the ends of very narrow conidiophores.

Conidia are solitary, smooth, one-celled, dark, oval, often flattened in side view, or spherical in surface view, often with a hyaline band around the margin and attached by a short connection to the conidiophore, and measure 5-6 x 3-4 µm.

Sterile cells, when present, are in place of conidia, and are usually smaller, paler and not the same shape as conidia.

Arthrinium is distinguished by the dark, one-celled, lentil shaped conidia with a pronounced hyaline (white) rim or germ slit.

Aspergillus flavus Link /*Aspergillus parasiticus* Spear

Disease

Storage rot of maize and wheat.
Aspergillus ear rot of maize.

Distribution

World-wide.

Significance

Crop production: Major cause of deterioration of maize stored above 15% moisture content. Drought and high temperatures favour the development of aflatoxins which are toxic to humans and animals, and reduce grain palatability for feed or food. Seed infection can reduce seed germination.

Quarantine: None known.

Note: The production of large numbers of air-disseminated spores can cause respiratory diseases in man and animals.

Identification

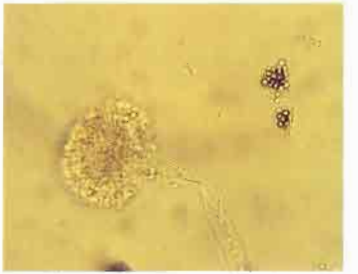
90

Colony on maize (x8)

91

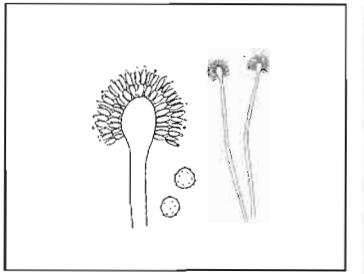
Colony on maize (x32)

92



Conidiophore and conidia (x400)

Quick clue



Detection technique

Freezing blotter method
(See Annex A)

References

CMI. 1966. *Descriptions of Pathogenic Fungi and Bacteria No. 91. Aspergillus flavus.* CAB, UK.

McGee, D.C. 1988. *Maize Diseases - A Reference Source for Seed Technologists.* APS Press, USA.

Malone, J.P., and Muskett, A.E. 1964. Seed-borne fungi - description of 77 fungus species. *Proc. Int. Seed Test. Assoc.* 29 (2): 179-384.

Raper, K.B., and Fennell, D.I. 1973. *The Genus Aspergillus.* Robert E. Kreiger Publishing Co., USA.

Colony on seed is usually spreading and very light yellow green, deep yellow green, olive-brown, or brown in colour.

Conidiophores are swollen apically and bear numerous conidia bearing cells (phialides) with conidia in long dry chains.

Conidial heads are typically spherical, splitting into several poorly defined columns, rarely exceeding 500-600 µm diameter, but mostly 300-400 µm.

Note: Severely infected wheat and maize seeds are discoloured and shrivelled.

Conidiophores are heavy walled, uncoloured, coarsely roughened, and usually less than 1 mm in length, with 10-20 µm diameter just below the apex.

Apices are elongated when young, becoming subspherical to spherical, and varying from 10-65 µm in diameter, but commonly 25-45 µm. There can be one or two series of conidia bearing cells (phialides and supporting cells) depending on the species.

Supporting cells are usually 6-10 x 4-6 µm but sometimes up to 15-16 x 8-9 µm in diameter.

Phialides measure 6-10 x 3-5 µm.

Conidia are typically spherical to subspherical, conspicuously spiny, variable, 3-6 µm diameter, and sometimes oval or pear-shaped at first and occasionally remaining so.

Aspergillus flavus/parasiticus species are recognised by their production of compact, spherical or columnar spore heads in some shade of very light yellow-green, deep yellow-green, olive-brown, or brown.

A. parasiticus has deeper green colonies, only phialides, spherical heads, and conidia which tend to be smaller and more delicately spiny.

Aspergillus niger van Tieghem

Disease

Storage rot of maize and wheat. Black mould; aspergillus ear rot of maize.

Distribution

World-wide.

Significance

Crop production: Major cause of deterioration of wheat and maize stored above 15% moisture content. Seed germination is reduced.

Quarantine: None known.

Note: The production of large numbers of air-disseminated spores can cause respiratory diseases in man and animals.

Identification

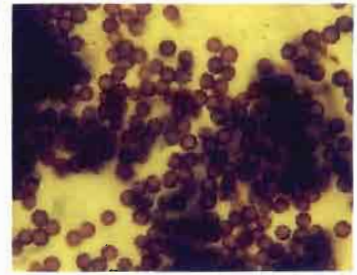
93

Colony on wheat (x8)

94

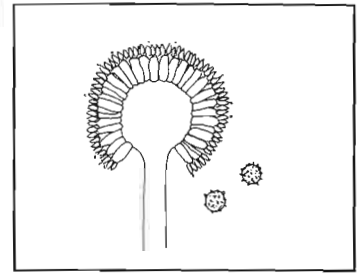
Colony on maize (x8)

95



Conidia (x1000)

Quick clue



Detection technique

Freezing blotter method
(See Annex A)

References

CMI. 1966. *Descriptions of Pathogenic Fungi and Bacteria No. 94. Aspergillus niger.* CAB, UK.

McGee, D.C. 1988. *Maize Diseases - A Reference Source for Seed Technologists.* APS Press, USA.

Raper, K.B., and Fennell, D.I. 1973. *The Genus Aspergillus.* Robert E. Kreiger Publishing Co., New York.

Colony on seed grows slowly, consisting of a compact to fairly loose white to faintly yellow basal mycelium, which bears abundant erect and usually crowded conidial structures, typically carbon black but sometimes deep brown black, covering the entire colony except for a narrow growing margin.

Conidial heads are typically large and black, compact at first, spherical, or split into two or more loose to reasonably well-defined columns, and commonly reach 700-800 µm in diameter.

Note: Severely infected wheat and maize seeds are discoloured and shrivelled.

Conidiophores are smooth, hyaline or faintly brownish near the apex and up to 3.0 mm in length and 15 - 20 µm in diameter.

Apices are spherical or nearly so, up to 75 µm in diameter but often quite small.

Two series of conidia bearing cells (supporting cells and phialides) are produced, but in some heads only phialides are present.

Supporting cells are of varying lengths and sometimes septate, but when mature usually 20-30 µm long.

Phialides are more uniform in length, usually 7-10 x 2-3 µm.

Conidia are typically spherical at maturity, often very rough or spiny, mostly 4-5 µm diameter, and very dark in colour or with conspicuous longitudinal striations.

Aspergillus niger is recognised by the production of compact, spherical or columnar spore heads in some shade of black - greenish black, brownish black, purplish black, or carbon black.

Botrytis Pers.

Disease
Botrytis stalk rot of maize.

Distribution
World-wide.

Significance
Crop production: Pathogen is possibly a secondary invader of maize stalks, and is of no economic importance.

Quarantine: None known.

Detection technique
Freezing blotter method
(See Annex A)

Identification

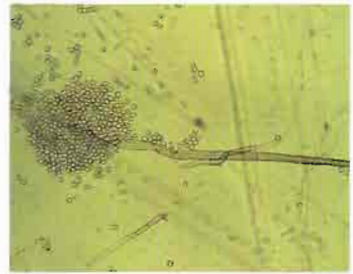
96

Colony on maize (x8)

97

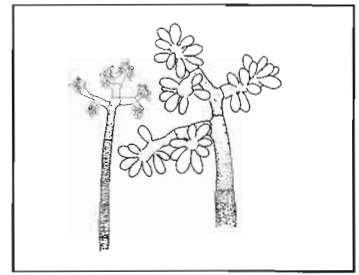
Colony on maize (x50)

98



Conidiophore and conidia (x160)

Quick clue



References

Barnett, H.L., and Hunter, B.B. 1972. *Illustrated Genera of Imperfect Fungi.* Burgess Publishing Co., USA.

Barron, G.L. 1972. *The Genera of Hyphomycetes from Soil.* R.E. Kreiger Publishing Co., New York, USA.

Ellis, M.B. 1971. *Dematiaceous Hyphomycetes.* CMI, UK.

Malone, J.P., and Muskett, A.E. 1964. Seed-borne fungi - description of 77 fungus species. *Proc. Int. Seed Test. Assoc.* 29 (2): 179-384.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists.* APS Press, USA.

Colony on seed is white or grey or greyish-brown, and spreading for a short distance around the affected seed.

Conidiophores are brown, tall, upright or nearly so, septate and branched, up to 30 µm wide and 2 mm long. The branches are constricted at their point of origin and quickly collapse when removed from a moist atmosphere.

Conidia occur in clusters at the swollen rounded apices and at intervals along the conidiophore on short blunt teeth. Conidia are oval or egg-shaped, often with a slightly projecting point of attachment, colourless to pale brown, and measure 6-18 x 4-11 µm.

Fairly large black irregular sclerotia can be produced, but not normally within the period of a seed health test. They are rather flat in appearance and measure 5 x 2 x 2 µm.

Botrytis is characterised by stout, brown, branched conidiophores supporting glistening grey heads of pale conidia, which can be observed under the low-power binocular microscope.

Cladosporium Link

Disease

Cladosporium ear rot (*C. herbarum, C. cladosporioides*) of maize. Black (sooty) head moulds (*C. herbarum, C. macrosporum*) of wheat.

Distribution

World-wide.

Significance

Crop production: Many saprophytic species are commonly encountered on maize and wheat seeds. Cladosporium ear rot of maize is of minor importance and usually associated with frost damage and wet weather. Black head moulds of wheat are caused by saprophytic or weakly parasitic species and are usually associated with insect infestations, lodging, nutrient deficiencies, and/or wet weather at maturation and harvest.

Identification

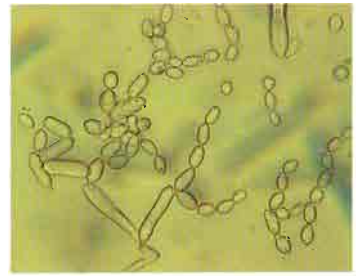
99

Colony on barley (x8)

100

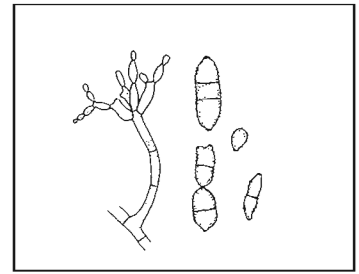
Colony on maize (x8)

101



Conidiophore and conidia (x1000)

Quick clue



Quarantine: None known.

Detection technique

Freezing blotter method
(See Annex A)

References

Barron, G.L. 1972. *The Genera of Hyphomycetes from Soil.* R.E. Kreiger Publishing Co., USA.

Ellis, M.B. 1971. *Dematiaceous Hyphomycetes.* CMI, UK.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists.* APS Press, USA.

Wiese, M.V. 1977. *Compendium of Wheat Diseases.* APS Press, USA.

Colony on seed spreading loosely or occasionally small point-like, cushion like, cotton-like groups or tufts, or hairy. Colour of colony is often olive green but also sometimes grey, light brownish yellow, brown or dark blackish brown. Colonies are relatively slow growing and produce little aerial mycelium but normally sporulate freely. Conidiophores are produced in dense stands from the seed.

Note: Heavily infected maize seeds may have dark-green to black blotches, or streaks that extend from the kernel tips.

Conidiophores are erect, pale olive-brown to brown, branched irregularly at the apex, tree-like, up to 250 µm long, 3-4 µm wide at the base, and tapering slightly towards the tip. Conidia are produced in chains, on conidiophore branches.

Conidia are oval, cylindrical or oblong with rounded ends, hyaline or olive-brown to brown, smooth or roughened, unicellular or 1-3 septate, and measure 5-23 x 3-8 µm. Conidial chains are very fragile, breaking up readily; fragmentation at maturity frequently involving the branches, and leaving only naked stumps of conidiophores entire.

Cladosporium is characterised by erect, pigmented conidiophores with chains of conidia in tree-like heads. This genus can frequently be identified by the distinctive lemon-shaped conidia, which have well marked, dark attachment scars and show considerable variation in size and septation within and between species.

Tree-like heads of conidiophores can be readily observed by using the scotch-tape method (see Annex A) under the microscope at low power (x100).

Epicoccum nigrum Link

Epicoccum purpurascens Ehrenb.

Disease

Red kernel disease of sweet corn.

Distribution

World-wide.

Significance

Crop production: Negligible impact; common saprophyte and secondary invader.

Quarantine: None known.

Detection technique

Freezing blotter method
(See Annex A)

Identification

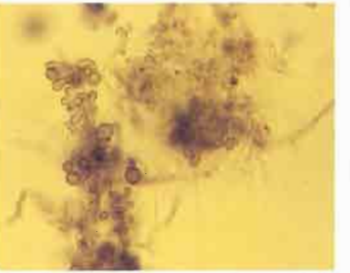
102

Colony on wheat (x8)

103

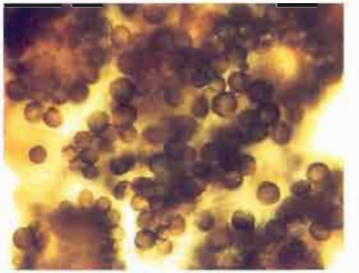
Colony on maize (x8)

104



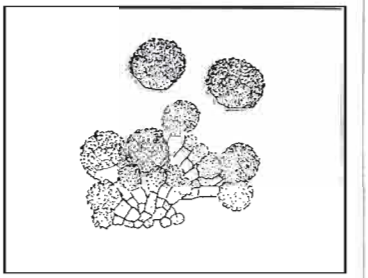
Conidiophores and conidia (x400)

105



Conidia (x400)

Quick clue



References

CMI. 1980. *Descriptions of Pathogenic Fungi and Bacteria No. 680, Epicoccum purpurascens*. CAB, UK.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists*, APS Press, USA.

Schol-Schwarz, M.B. 1959. The genus *Epicoccum* Link. *Trans. Brit. Mycol. Soc.* 42(2):149-173.

Colony on seed grows rapidly, often producing a yellow, amber to orange, or red pigmentation within but particularly surrounding the white, compact mycelium.

Epicoccum is occasionally confused with *Fusarium* spp. due to these features.

Note: Infected maize seeds may be coloured red.

Conidiophores are compact or occasionally branched, loose, dark, smooth, short, occurring in tight clusters from the hyphae and produce a single terminal conidium.

Mature conidia are dark brown to black, mostly spherical but also pear-shaped, irregularly septate, and may appear to be very coarsely marked like a net. The septa are often hidden by the thick, rough spore wall which appears to be covered by short, blunt projections. Conidia measure 15-25 µm in diameter and most often occur in dark, cushion shaped spore masses of variable size within and on the surface of the mycelium.

Colonies of *Epicoccum* species are often variable in colour, and a number of shades may be present in any colony; those which occur most often are yellow, orange and red, and, occasionally, brown and green. If present, dark spore masses may look like black sand sprinkled over the mycelium.

Individual spores resemble dark, rough soccer balls, and may be confused with spores of smuts and bunts.

Gonatobotrys Corda

Gonatobotrys simplex Corda

Gonatobotrys zeae Futrell & Bain (nomen nudum)

Disease

Gonatobotrys seed rot of maize (*G. zeae*). None on wheat.

Distribution

World-wide.

Significance

Crop production: No definitive evidence that *G. zeae* causes a disease of maize. None on wheat.

Quarantine: None known.

Note: *G. simplex* is a parasite on *Alternaria* spp. and *Cladosporium* spp.

Detection technique

Freezing blotter method
(See Annex A)

Identification

106

Colony on maize with *F. oxysporum* (x8)

107

Colony on wheat (x50)

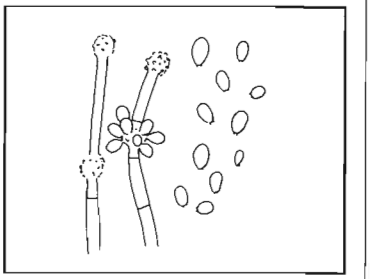
108

Conidiophore and conidia (x400)

109

Conidiophore and conidia (x1000)

Quick clue



References

Barnett, H.L., and Hunter, B.B. 1972. *Illustrated Genera of Imperfect Fungi*. Burgess Publishing Co., USA.

Barron, G.L. 1972. *The Genera of Hyphomycetes from Soil*. R.E. Kreiger Publishing Co., New York, USA.

Futrell, C., and Bain, D.C. 1968. *Gonatobotrys zeae* sp. n., a new pathogen on corn. (Abstract). *Phytopathology* 58 (6): 728.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists*. APS Press, USA.

Whaley, J.W., and Barnett, H.L. 1963. Parasitism and nutrition of *Gonatobotrys simplex*. *Mycologia* 55 (2): 199-210.

Colony on seed is white and usually on the surface of another fungal species e.g. *Alternaria*, *Cladosporium* and *Fusarium*. Mycelium appears as a mass of strings with clusters of 'flower like' bunches of conidia.

Conidiophores are erect, sometimes tall, septate, simple or occasionally branched, with inflated cells covered with a series of blunt teeth bearing conidia, inserted at intervals and terminally on the hyphae.

Conidia, borne singly on the blunt teeth, are 1-celled, hyaline, oval to subspherical, and measure 10-22 x 6-12 µm.

Gonatobotrys is distinguished by the clusters of large, hyaline, conidia arising from "nodes" along the length of the conidiophores, with the appearance of a 'string of beads'.

Monilia Pers.

Disease
None.

Distribution
World-wide.

Significance
Crop production: Negligible impact; common saprophyte.

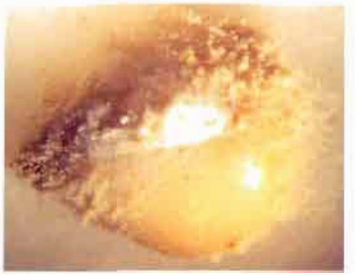
Quarantine: None known.

Note: *Monilia sitophila*, the red bread mould, has a very rapid growth rate and can be a nuisance as a contaminant of seed health tests.

Detection technique
Freezing blotter method
(See Annex A)

Identification

110

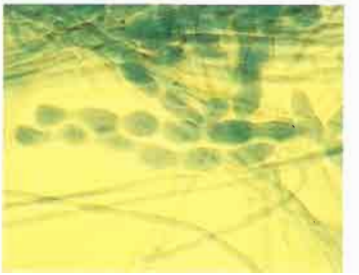


Colony on maize (x8)

111

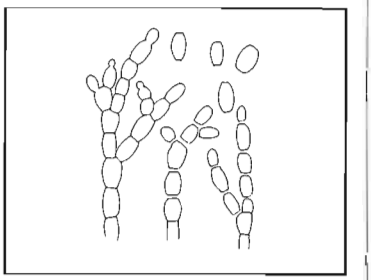
Colony on maize (x20)

112



Conidiophores and conidia (x400)

Quick clue



References

Barnett, H.L., and Hunter, B.B. 1972. *Illustrated Genera of Imperfect Fungi.* Third Edition. Burgess Publishing Co., USA.

Barron, G.L. 1972. *The Genera of Hyphomycetes from Soil.* R.E. Kreiger Publishing Co., New York, USA.

von Arx, J.A. 1981. On *Monilia sitophila* and some families of ascomycetes. *Sydowia* 34: 13-29.

Colony on seed is cream to apricot coloured, with loose, white or grey mycelium and rapid growth.

Conidiophores are little differentiated from the white or grey vegetative hyphae. They are erect or straggling, simple or irregularly branched, hyaline, and septate.

Conidia are produced in apical succession to give branched chains. Conidia are hyaline to sub-hyaline, continuous, spherical to egg shaped, and appear pink, grey or tan in mass.

Distinctive branched chains of hyaline conidia with a bead-like appearance breaking up readily to form conidia of irregular shape and size.

Myrothecium Tode

Disease
None.

Distribution
Widespread.

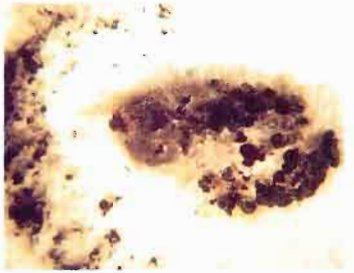
Significance
Crop production: Negligible impact. Common saprophyte.

Quarantine: None known.

Detection technique
Freezing blotter method
(See Annex A)

Identification

113

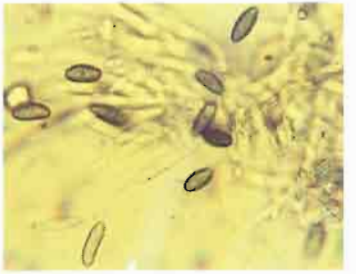


Colony on wheat (x8)

114

Colony on wheat (x32)

115

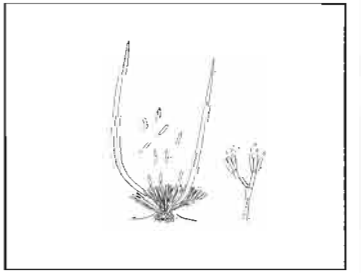


Conidiophores and conidia (x1000)

116

Conidia (x1000)

Quick clue



References

Preston, N.C. 1943. Observations on the genus *Myrothecium* Tode. I. The three classic species. *Trans. Brit. Mycol. Soc.* 26: 158-168.

Preston, N.C. 1948. Observations on the genus *Myrothecium* II. *Myrothecium gramineum* Lib. and two new species. *Trans. Brit. Mycol. Soc.* 31:271-276

Tulloch, M. 1972. The genus *Myrothecium* Tode ex Fr. *CMI Mycological Papers* 130: 1-42.

Colony on seed consists of rapidly growing white mycelium forming dark green spore masses on the seed surface and at the edge of the colony on the blotter paper.

Spore masses are formed from closely compacted conidiophores bearing a mass of slimy green to black conidia which becomes hard on drying.

Conidiophores are hyaline or olive-green or slightly darkened, irregularly and repeatedly branched forming several branches at each node, with the ultimate branches bearing the conidia in whorls.

Conidia are unicellular, flattened or lentil-shaped, hyaline or dilute olive green, black in mass, and slimy.

Dark olive green spore masses bearing a mass of slimy green to black unicellular, flattened or lentil-shaped conidia.

Nigrospora Zimm.

Disease
Ear and stalk rot of maize.

Distribution
World-wide.

Significance
Crop production: Common occurence but minor losses. Ear rot is of more importance than stalk rot.

Quarantine: None known.

Note: Saprophyte on plant debris in warmer areas.

Detection technique
Freezing blotter method
(See Annex A)

Identification

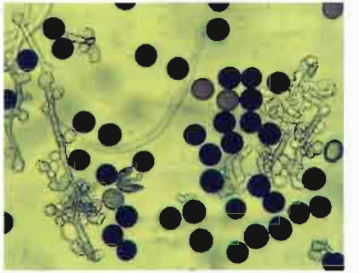
117

Colony on wheat (x8)

118

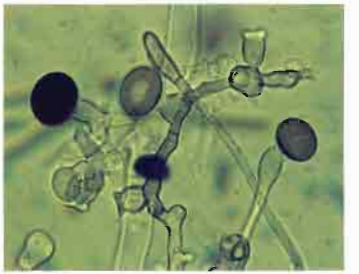
Colony on wheat (x32)

119



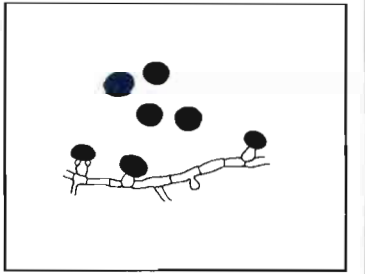
Conidiophores and conidia (x400)

120



Conidiophores
and conidia (x1000)

Quick clue



References

Barron, G.L. 1972. *The Genera of Hyphomycetes from Soil.* R.E. Kreiger Publishing Co., New York, USA.

CMI. 1971. Descriptions of Pathogenic Fungi and Bacteria No. 311, *Khuskia oryzae.* CAB, UK.

Hudson, H.J. 1963. The perfect state of *Nigrospora oryzae. Trans. Brit. Mycol. Soc.* 46 (3): 355-360.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists.* APS Press, USA.

Standen, J.H. 1945. *Nigrospora oryzae* (B. and Br.) Petch on Maize. *Phytopathology* 35: 552-564.

Colony on seed is initially white and the shiny, black conidia standing out in sharp contrast giving the colonies a striking appearance under the binocular dissecting microscope. In older cultures the hyphae darken and the colonies appear black, with profuse conidia production.

Note: Infected seeds have white streaks with black spore masses near the tips.

Conodiophores are short, pale brown, inflated and borne at right angles to hyphae; bearing conidia singly and terminally.

Conidia are smoky brown or jet black, spherical or egg-shaped, 10-16 x 10-13 µm, and commonly measure 12-14 µm in diameter.

Perithecia, formed in clusters of 1-7 in series or irregular rows, up to 2 mm long, are spherical or oval, and up to 250 µm in diameter with protruding pore openings.

Asci are short-stalked, club-shaped, and measure 55-75 x 8-12 µm with 8 ascospores.

Ascospores are hyaline, granular, curved, 16-21 x 5-7 µm, and tapering to the base with rounded ends. They are initially one-celled but after discharge from ascus may develop a single transverse septum dividing the spore unequally into two-cells.

Very dark conidia, which are slightly longer in the horizontal axis and borne on very short pale brown conidiophores with a characteristic bulge.

Papulaspora Preuss

Disease
None.

Distribution
World-wide.

Significance
Crop production: None. Common saprophyte and secondary invader.

Quarantine: None known.

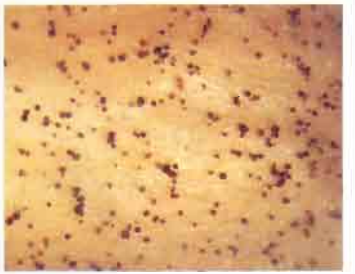
Detection technique
Freezing blotter method (See Annex A)

Identification

121

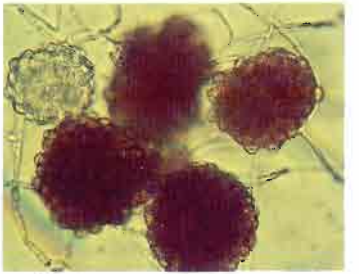
Colony on maize (x8)

122



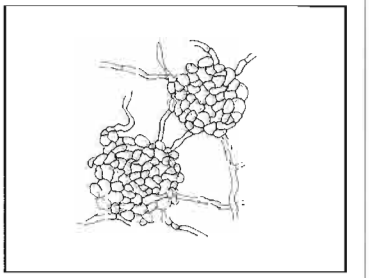
Colony on maize (x32)

123



Bulbils (x400)

Quick clue



References

Barnett, H.L., and Hunter, B.B. 1972. *Illustrated Genera of Imperfect Fungi.* Third Edition. Burgess Publishing Co., USA.

Barron, G.L. 1972. *The Genera of Hyphomycetes from Soil.* R.E. Kreiger Publishing Co., New York, USA.

Malone, J.P., and Muskett, A.E. 1964. Seed-borne fungi - description of 77 fungus species. *Proc. Int. Seed Test. Assoc.* 29 (2): 179-384.

Colony on seed is initially of dispersed, fine, cobwebby, white aerial mycelium, becoming brown or red in colour due to the formation of bulbils in the mycelium.

Bulbils are compact irregular clusters of small cells, formed by the coiling of short lateral branches of the hyphae, the cells of which multiply and become enlarged. Bulbils are pale orange, red or brown and serve to reproduce the fungus. Some bulbils consist of a core of one or more darker cells surrounded by lighter coloured ones while others appear to be uniformly coloured. They are almost spherical or oval in shape and measure 20-130 µm in diameter depending on the species.

Conidiophores and conidia are lacking. In this genus no true spores are formed.

Papulaspora is distinguished by the characteristic 'bulbils' (compact irregular clusters of small cells) borne by the hyaline vegetative hyphae.

Penicillium Link

Disease

Ear rot of maize. Seed rot of wheat. Storage mould of wheat and maize.

Distribution

World-wide.

Significance

Crop production: Minor losses from ear rot of maize and seed rot of wheat. However, rotting of maize and wheat grain at elevated moisture and temperature regimes can be significant. Reduced germination and seedling blight, particularly of sweet corn seed, are important.

Quarantine: None known.

Note: Grain mycotoxins are of minor concern.

Identification

124

Colony on wheat (x8)

125

Colony on maize (x8)

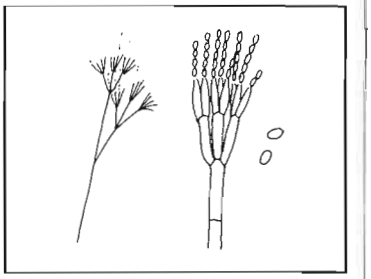
126

Conidiophores (x64)

127

Conidiophore and conidia (x1000)

Quick clue



Detection technique

Freezing blotter method
(See Annex A)

References

Caldwell, R.W., Tuite, J., and Carlton, W.W. 1981. Pathogenicity of *Penicillia* to Corn Ears. *Phytopathology* 71 (2): 175-180.

Malone, J.P., and Muskett, A.E. 1964. Seed-borne fungi - description of 77 fungus species. *Proc. Int. Seed Test. Assoc.* 29 (2): 179-384.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists*. APS Press, USA.

Wiese, M.V. 1977. *Compendium of Wheat Diseases*. APS Press, USA.

Colonies on seed have a slow to moderate rate of growth. Mycelium is usually not very plentiful but sporulation takes place freely giving a colony with a cushion-like appearance, which is usually some shade of blue/green.

Note: Infected seeds may have white streaks.

Conidiophores are usually conspicuous, more or less erect, hyaline, rough or smooth, septate, with a series of branches giving a characteristic brush-like structure with typically flask-shaped, hyaline phialides producing long dry chains of conidia. The 'brush' may consist of a single whorl of phialides or a series of branches in whorls, each ending in a whorl of phialides.

Conidia are one-celled, spherical or oval, smooth or roughened, and brightly coloured - usually a shade of blue/green.

Sclerotia are formed in some species.

Long conidiophores that branch in a broom-like fashion with flask-shaped phialides bearing long chains of abundant conidia. The conidia are spherical to oval, and under the microscope resemble glass beads.

Penicillium colonies may be confused with those of *Aspergillus* spp. However, the spore masses of *Penicillium* are usually blue/green and conidia of *Aspergillus* are borne on globe-like rather than broom-like structures.

Rhinotrichum Corda (*Oidium* Link)

Disease
None.

Distribution
Widespread.

Significance
Crop production: None. Common saprophyte and secondary invader.

Quarantine: None known.

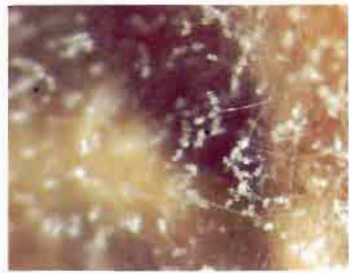
Detection technique
Freezing blotter method
(See Annex A)

Identification

128

Colony on maize (x8)

129

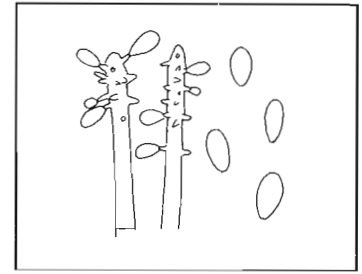


Colony on maize (x64)

130

Conidiophores and conidia (x400)

Quick clue



References

Barnett, H.L., and Hunter, B.B. 1972. *Illustrated Genera of Imperfect Fungi*. Third Edition. Burgess Publishing Co., USA.

Barron, G.L. 1972. *The Genera of Hyphomycetes from Soil*. R.E. Kreiger Publishing Co., New York, USA.

Colony on seed is slow growing with sparse or dense white cotton wool-like mycelium.

Conidiophores are stout, more or less erect, septate, simple or branched with spore-bearing cells at the ends of branches and the main axis.

Spore-bearing cells are cylindrical, sometimes enlarged, with prominent blunt teeth.

Conidia are 1-celled, spherical to oval, smooth, hyaline or slightly coloured, and produced singly with the growing point at the tip.

Large, hyaline or subhyaline, spherical to oval conidia borne on pronounced blunt teeth at the apex of stout conidiophores are typical.

Stachybotrys Corda

Disease
None.

Distribution
World-wide.

Significance
Crop production: None. Common saprophyte and secondary invader.

Quarantine: None known.

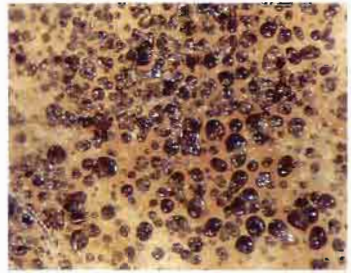
Detection technique
Freezing blotter method
(See Annex A)

Identification

131

Colony on maize and blotter (x8)

132

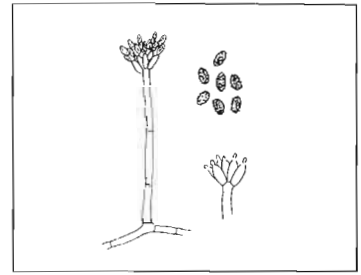


Colony on blotter (x64)

133

Conidiophores and conidia (x1000)

Quick clue



References

Barron, G.L. 1972. *The Genera of Hyphomycetes from Soil.* R.E. Kreiger Publishing Co., New York, USA.

Bisby. G.R. 1943. *Stachybotrys.* Trans. Brit. Mycol. Soc. 26: 133-143.

IMI. 1991. Descriptions of Pathogenic Fungi and Bacteria No. 1060 . *Stachybotrys atra.* CABI, UK.

Ellis, M.B. 1971. *Dematiaceous Hyphomycetes.* CMI, UK.

Malone, J.P., and Muskett, A.E. 1964. Seed-borne fungi - description of 77 fungus species. *Proc. Int. Seed Test. Assoc.* 29 (2): 179-384.

Colony on seed has little aerial mycelium which is at first white, then grey and finally becoming black with the production of conidia.

Conidiophores and conidia are often formed on the blotter paper around the seed, giving the appearance of black or blackish green glistening patches.

Conidiophores arise from the mycelium, without foot cells, and are rarely branched near the base, erect, septate, dark coloured, up to 100 µm high, 3-5 µm wide, with the upper portion often being roughened and darker in colour.

Phialides are dark, club-shaped, and approximately the same size as the conidia (10-13 µm long and 4-6 µm wide). Phialides arise in groups of 3-7 from the tip of each conidiophore, and remain attached to the conidiophore when the conidia are removed.

Conidia occur in compact balls held together by mucilage. They are oval to cylindrical with rounded ends, dark coloured, smooth-walled or minutely roughened, (especially when older), and measure 8-11 µm long, by 5-10 µm wide.

The cluster of several swollen phialides at the apex of a simple conidiophore is characteristic of *Stachybotrys.*

In most of the common species of *Stachybotrys*, the dark conidia slime down to form glistening heads.

Stemphylium Wallr.

Teleomorph: *Pleospora* Rabenh. ex Ces. & de Not.

Disease

Black (sooty) head mould of wheat.

Distribution

World-wide.

Significance

Crop production: Negligible impact. Common saprophyte on dead plant material.

Quarantine: None known.

Detection technique

Freezing blotter method
(See Annex A)

Identification

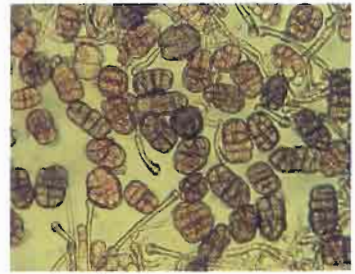
134

***Stemphylium* colony on barley (x64)**

135

***Pleospora* colony on barley (x64)**

136

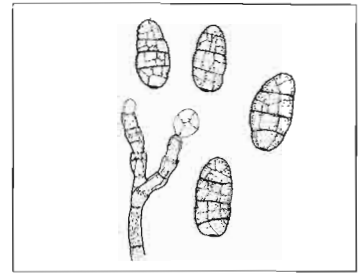


Conidia (x400)

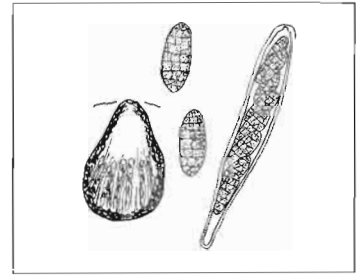
137

Ascostromata and asci (x160)

Quick clue



Stemphylium



Pleospora

References

Barron, G.L. 1972. *The Genera of Hyphomycetes from Soil*. R.E. Kreiger Publishing Co., New York, USA.

CMI. 1967. Descriptions of Pathogenic Fungi and Bacteria No. 150, *Pleospora herbarum*. CAB, UK.

Ellis, M.B. 1971. *Dematiaceous Hyphomycetes*. CMI, UK.

Simmons, E.G. 1967. Typification of *Alternaria*, *Stemphylium*, and *Ulocladium*. *Mycologia* 59:67-92.

Wiese, M.V. 1977. *Compendium of Wheat Diseases*. APS Press, USA.

Zillinsky, F.J. 1983. *Common Diseases of Small Grain Cereals*. Mexico, CIMMYT.

Stemphylium colony on seed is grey, brown, olive brown or black in colour and, cushion-like or cottony in appearance. It grows fairly rapidly, consisting of conidiophores and numerous brown/black conidia.

Pleospora colony on seed has dispersed, thread-like, hyaline to brown aerial mycelium, with the production of relatively large black spherical or somewhat flattened ascostromata.

Conidiophores are dark brown, simple or sometimes branched, 3 to 6 µm in diameter, and develop dark successive terminal swellings as each new conidium is produced in succession.

Conidia are olive to dark brown, with horizontal, vertical and oblique cross walls, rectangular to oval in shape; rounded at ends, smooth or roughened, and often narrowed at one or more of the septa. Older conidia are almost black, coarsely rough and measure 15-20 x 18-35 µm.

Ascostromata are spherical or somewhat flattened, black, and 100-500 µm in diameter.

Asci are cylindrical to club-shaped, 90-250 x 20-50 µm with eight ascospores ranked in two rows.

Ascospores are light to dark yellow brown, club-shaped, 7-septate, 26-50 x 10-20 µm, with horizontal, vertical and oblique cross walls.

Stemphylium is identified by conidiophores with dark, terminal swellings at intervals along the hyphae, bearing solitary, pigmented conidia with horizontal, vertical and oblique cross walls.

Conidia lack the prominent beak that characterizes *Alternaria* species.

Pleospora is identified by ascostromata which are large, black, spherical or somewhat flattened with club-shaped asci containing eight 7-septate ascospores with horizontal, vertical and oblique cross walls.

Torula Pers.

Disease
Sooty head mould of wheat.

Distribution
World-wide.

Significance
Crop production: None. Common saprophyte and secondary invader.

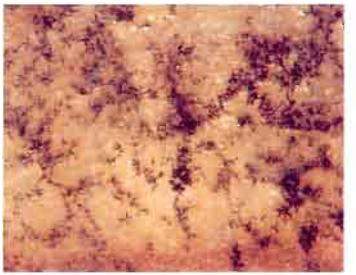
Quarantine: None known.

Note: More predominant during wet harvests.

Detection technique
Freezing blotter method
(See Annex A)

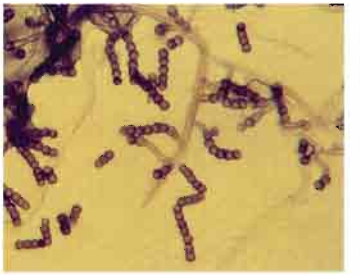
Identification

138



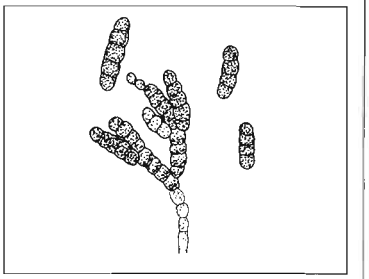
Colony on maize (x64)

139



Conidiophores and conidia (x400)

Quick clue



References

Barron, G.L. 1972. *The Genera of Hyphomycetes from Soil.* R.E. Kreiger Publishing Co., New York, USA.

Malone, J.P., and Muskett, A.E. 1964. Seed-borne fungi - description of 77 fungus species. *Proc. Int. Seed Test. Assoc.* 29 (2): 179-384.

Zillinsky, F.J. 1983. *Common Diseases of Small Grain Cereals.* Mexico, CIMMYT.

Colony on seed forms small, compact, olive green mounds which may coalesce and when older tend to become brown.

Conidiophores are short or lacking, and not readily distinguished, with conidia arising more or less directly from the vegetative hyphae.

Conidia develop in long chains, which break into segments of from one to many cells when mature.

Conidia are barrel shaped with the end cells rounded, about 6 µm in diameter, smooth to minutely rough surface and dark brown to black in colour. Branching of the chain often takes place at a spherical cell which is darker and more distinctly spiny than the others.

Torula is characterized by simple or branched chains of dark conidia which break up readily and which arise more or less directly from the vegetative hyphae.

Trichoderma Pers.

Disease
Ear rot of maize.

Distribution
Widespread.

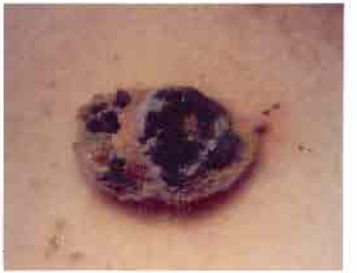
Significance
Crop production: Negligible impact. Often secondary invader after severe leaf damage by other fungi or *Bipolaris maydis* ear infection.

Quarantine: None known.

Detection technique
Freezing blotter method
(See Annex A)

Identification

140



Colony on wheat (x8)

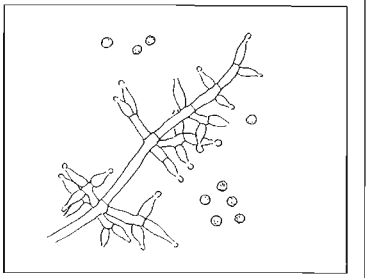
141

Colony on maize (x8)

142

Conidiophores and conidia (x1000)

Quick clue



References

Barron, G.L. 1972. *The Genera of Hyphomycetes from Soil.* R.E. Kreiger Publishing Co., New York, USA.

Malone, J.P., and Muskett, A.E. 1964. Seed-borne fungi - description of 77 fungus species. *Proc. Int. Seed Test. Assoc.* 29 (2): 179-384.

Shurtleff, M.C. 1980. *Compendium of Corn Diseases.* APS Press, USA.

Colony on seed forms small white mounds which coalesce and soon become green with spore masses.

Note: Infected maize ears can have green woolly mycelia on and between the seeds.

Conidiophores are hyaline, septate, erect or straggling, and solitary or frequently branched more or less at right angles to the main axis.

Conidia-bearing phialide cells are hyaline, oval to flask-shaped, and measure 5-15 x 3-4 µm. Phialides arise singly, in pairs, or in clusters and are frequently borne at right angles to the parent conidiophore or branch, with clusters in pairs or alternately.

Conidia are hyaline or green, smooth, or rough, spherical to egg-shaped, 3-4 µm in diameter, and non-septate. Conidia gather in small spherical green balls consisting of 10 to 20 conidia, each on the phialides.

Very distinctive colony on seed with rapidly growing, cotton-like groups or tufts with cushions of conidia, which are white, yellow-green or bright green.

Trichoderma often parasitizes and overgrows other fungi present.

Trichothecium roseum Link

Disease
None.

Distribution
Widespread.

Significance
Crop production: None. Common saprophyte and secondary invader.

Quarantine: None known.

Detection technique
Freezing blotter method
(See Annex A)

Identification

143

Colony on wheat (x10)

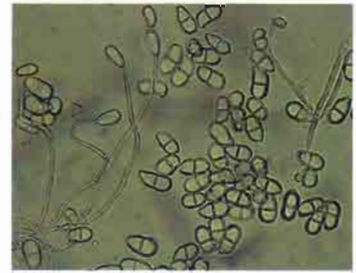
144

Colony on maize (x8)

145

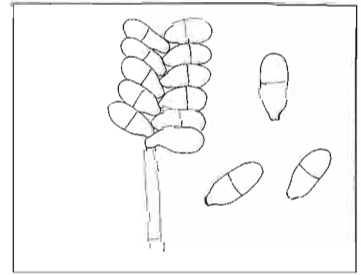
Conidiophores (x32)

146



Conidiophores and conidia (x400)

Quick clue



References

Barnett, H.L., and Hunter, B.B. 1972. *Illustrated Genera of Imperfect Fungi.* Third Edition. Burgess Publishing Co., USA.

Barron, G.L. 1972. *The Genera of Hyphomycetes from Soil.* R.E. Kreiger Publishing Co., New York, USA.

Malone, J.P., and Muskett, A.E. 1964. Seed-borne fungi - description of 77 fungus species. *Proc. Int. Seed Test. Assoc.* 29 (2): 179-384.

Rifai, M.A., and Cooke, R.C. 1966. Studies on some didymosporous genera of nematode-trapping hyphomycetes. Trans. Br. Mycol. Soc. 49 (1): 147-168.

Colony on seed usually appears as a salmon pink crust with the production of numerous conidia. Colonies can be cushion-like or powdery.

Conidiophores are erect or suberect, produced singly or in groups, simple or sparingly branched, long, slender, hyaline, and septate. Conidia are produced in short, fragile chains.

Conidia are large (12-18 x 8-10 µm), smooth, two-celled (slightly narrowed at the septum), hyaline, more or less egg-shaped, with well marked attachment point and upper cell somewhat larger than the lower one.

The short chains of two-celled conidia at the apex of a hyaline, simple conidiophore are diagnostic.

Colony on seed superficially resembles the spore masses of *Fusarium* or *Gliocladium* species.

Ulocladium Preuss

Disease
None.

Distribution
Widespread.

Significance
Crop production: None. Common saprophyte and secondary invader.

Quarantine: None known.

Detection technique
Freezing blotter method (See Annex A)

Identification

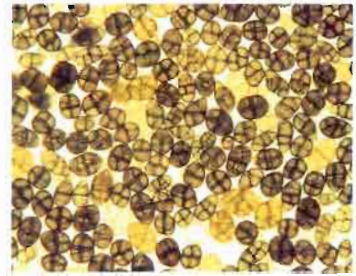
147

Colony on wheat (x8)

148

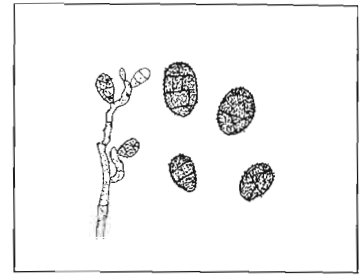
Colony on maize (x8)

149



Conidia (x400)

Quick clue



References

Barnett, H.L., and Hunter, B.B. 1972. *Illustrated Genera of Imperfect Fungi.* Third Edition. Burgess Publishing Co., USA.

Barron, G.L. 1972. *The Genera of Hyphomycetes from Soil.* R.E. Kreiger Publishing Co., New York, USA.

Ellis, M.B. 1971. *Dematiaceous Hyphomycetes.* CMI, UK.

Simmons, E.G. 1967. Typification of *Alternaria, Stemphylium*, and *Ulocladium. Mycologia* 59:67-92.

Colony on seed dark blackish brown to black, and cushion-like.

Conidiophores arising as upright branches of mycelium are pale to mid golden brown, mostly without branches, smooth, septate, up to 100 µm long, and 3-5 µm thick.

Conidia are golden brown, variable in shape, slightly rough, mostly egg-shaped, not beaked, with 1-3 horizontal septa and 1 or more vertical cross walls, and usually without narrowing at major septum. They are borne singly or successively towards the apex with the youngest conidia at the apex, and measure 13-30 x 6-19 µm.

Tips of conidiophores are not markedly swollen as in *Stemphylium.*

Conidia are distinctly egg-shaped with the narrow end attached to the conidiophore; in contrast with *Alternaria* sp. where similar shaped conidia are attached to the conidiophore at the wide base.

Conidia also lack the prominent beak that characterizes *Alternaria* species.

Verticillium Nees

Disease
None.

Distribution
Widespread but predominantly in temperate regions.

Significance
Crop production: None.
Common secondary invader.

Quarantine: None known.

Detection technique
Freezing blotter method
(See Annex A)

Identification

150

Colony on wheat (x8)

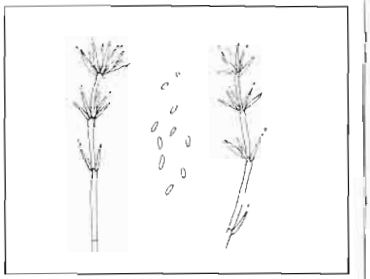
151

Colony on wheat (x64)

152

Conidiophores and conidia (x400)

Quick clue



References

Barnett, H.L., and Hunter, B.B. 1972. *Illustrated Genera of Imperfect Fungi.* Third Edition. Burgess Publishing Co., USA.

CMI. 1970. Descriptions of Pathogenic Fungi and Bacteria No. 255 . *Verticillium albo-atrum.* CAB, UK.

Malone, J.P., and Muskett, A.E. 1964. Seed-borne fungi - description of 77 fungus species. *Proc. Int. Seed Test. Assoc.* 29 (2): 179-384.

Colony on seed is white to greyish with cotton-like mycelium in tufts.

Conidiophores are abundant, more or less erect, hyaline, septate, variable in length, and characteristically darkened at the base when growing on plant tissue. Conidia-bearing phialides are arranged in whorls (2-4 phialides) at the tip and at intervals along the length, or singly or in groups laterally on the hyphae; and are sometimes secondarily branched.

Phialides are hyaline, variable in size, mainly 20-30 x 1-3 µm and tapering towards the tip where the conidia are formed in a globule.

Conidia arising singly at the apices of the phialides, are hyaline, cylindrical with rounded ends, sometimes oval, mainly simple, occasionally 1-septate, and measure 3-11 x 2-4 µm.

Verticillium is characterised by fine white mycelium and distinctive conidiophores with whorls of two to four phialides at the tip and at intervals along the length, bearing conidia in globules at their tips.

Colonies may be confused with *F. moniliforme* and *Acremonium* spp. by colony appearance and the abundance of small cylindrical conidia. However, they are differentiated by the dimensions of conidia and conidiophores and the arrangement of the conidia-bearing cells on the conidiophores.

Lasiodiplodia Ellis & Everh.

Botryodiplodia Sacc.

Disease

Black kernel rot of maize.

Distribution

World-wide between 40ºN and 40ºS of equator.

Significance

Crop production: Disease causes extensive losses in India. Severely infected seeds are rotted and fail to germinate.

Quarantine: None known.

Detection technique

Freezing blotter method
(See Annex A)

Identification

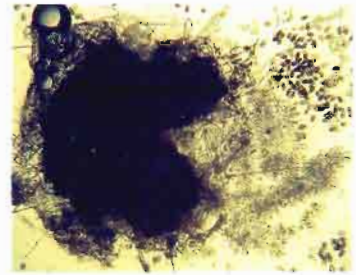
153

Colony on maize (x8)

154

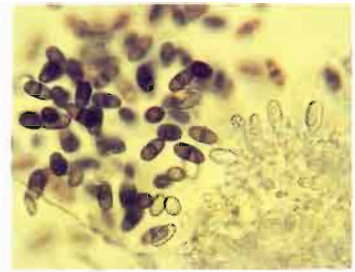
Colony on maize (x16)

155



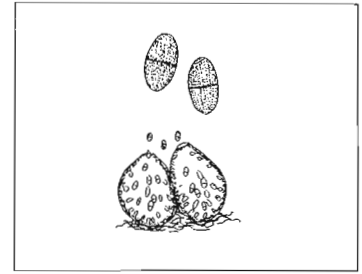
Pycnidia, conidiophores, and conidia (x100)

156



Conidiophores and conidia (x400)

Quick clue



References

CMI. 1976. Descriptions of Pathogenic Fungi and Bacteria No. 519. *Botryodiplodia theobromae.* CAB, UK.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists.* APS Press, USA.

Kumar, V., and Shetty, H.S. 1983. Seed-borne nature and transmission of *Botryodiplodia theobromae* in maize (*Zea mays*). *Seed Sci. and Technol.* 11: 781-789.

Shurtleff, M.C. 1980. *Compendium of Corn Diseases.* APS Press, USA.

Sutton, B.C. 1980. *The Coelomycetes - Fungi Imperfecti with Pycnidia, Acervuli and Stromata.* CMI, UK.

Colony on seed is initially white and gradually turns greyish-black and fluffy with abundant aerial mycelium. Black pycnidia occur singly or frequently in clumps surrounded by a dark mat of mycelium.

Note: Infected seeds are shiny-black or have grey-black streaks (with/without pycnidia).

Black pycnidia are oval to oblong, simple, or compound, with a pore opening, and up to 5mm in diameter.

Conidiophores are hyaline, simple or septate, short, cylindrical and rarely branched.

Conidia are oval, cinnamon to fawn brown, with longitudinal stripes, one-septate, and measure 10-15 x 20-30 µm.

Lasiodiplodia species are characterised by black, oval pycnidia in clumps with conidia exuding from the pore opening in a column. The column of conidia when yellowish in colour consists mostly of 1-celled conidia; but after 3-4 days it becomes black and consists mainly of 2-celled spores.

Pestalotiopsis Stey.

Disease
None.

Distribution
World-wide.

Significance
Crop production: None. Common saprophyte and secondary invader.

Quarantine: None known.

Detection technique
Freezing blotter method
(See Annex A)

Identification

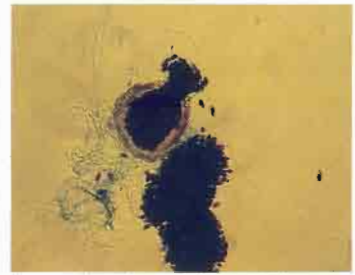
157

Colony on wheat (x8)

158

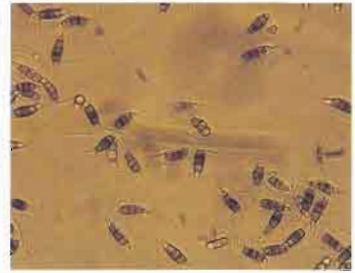
Colony on maize (x8)

159



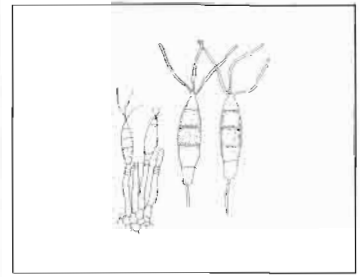
Acervuli and conidia (x160)

160



Conidia (x400)

Quick clue



References

Barnett, H.L., and Hunter, B.B. 1972. *Illustrated Genera of Imperfect Fungi.* Third Edition. Burgess Publishing Co., USA.

Barron, G.L. 1972. *The Genera of Hyphomycetes from Soil.* R.E. Kreiger Publishing Co., New York, USA.

CMI. 1980. Descriptions of Pathogenic Fungi and Bacteria No. 675, *Pestalotiopsis dichaeta.* CAB, UK.

Sutton, B.C. 1980. *The Coelomycetes - Fungi Imperfecti with Pycnidia, Acervuli and Stromata.* CMI, UK.

Colony on seed is white with occasional faint grey to yellowish areas in the dense aerial mycelium.

Acervuli develop from small clumps of hyphae and form conspicuous greenish black slimy spore masses.

Conidia emerge initially in black columns, and later become loosely spread over the colony surface.

Acervuli are dark, disc-shaped or cushion-shaped, and up to 300 µm in diameter.

Conidiophores are hyaline, cylindrical, short, and branched.

Conidia are narrowed towards the ends, straight or curved, 5-celled, and slightly narrowed at the septa. Conidia have hyaline (rarely dilutely coloured), pointed end cells, with two or more hyaline, apical appendages; intermediate cells equally or variably coloured, pale brown to almost black; and a hyaline, simple, rarely branched, basal appendage.

Pestalotiopsis is characterized by distinctive dark conidia, with hyaline end cells, and the apex bearing two or more bristle-like appendages.

Phoma Westend.

Disease

Minor leaf disease of wheat.
Stalk rot of maize.

Distribution

World-wide.

Significance

Crop production: May occur as a pathogen after prolonged periods of humid weather. Frequently observed as a secondary invader.

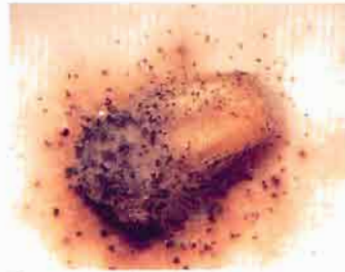
Quarantine: None known.

Detection technique

Freezing blotter method
(See Annex A)

Identification

161



Colony on wheat (x8)

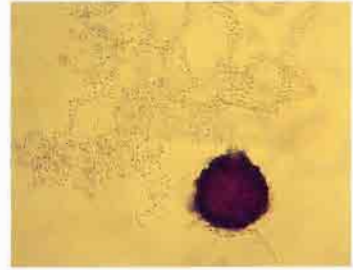
162

Colony on maize (x8)

163

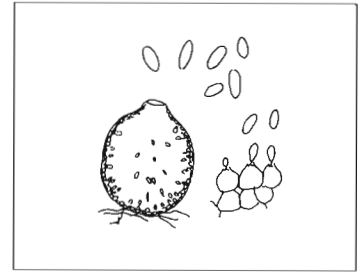
Pycnidia on maize (x64)

164



Pycnidia and conidia (x160)

Quick clue



References

Boerema, G.H. 1976. The *Phoma* species studied in culture by Dr. R.W.G. Dennis. *Trans. Brit. Mycol Soc.* 67 (2): 289-319.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists.* APS Press, USA.

Malone, J.P., and Muskett, A.E. 1964. Seed-borne fungi - description of 77 fungus species. *Proc. Int. Seed Test. Assoc.* 29 (2): 179-384.

Sutton, B.C. 1980. *The Coelomycetes - Fungi Imperfecti with Pycnidia, Acervuli and Stromata.* CMI, UK.

Wiese, M.V. 1977. *Compendium of Wheat Diseases.* APS Press, USA.

Zillinsky, F.J. 1983. *Common Diseases of Small Grain Cereals.* Mexico, CIMMYT.

Colony on seed has very little white/grey mycelium but produces large numbers of dark-brown or black pycnidia on the seed surface or the blotter paper.

Pycnidia are almost spherical, dark brown, thin-walled, and variable in size (100-300 µm diameter), with one conspicuous protruding pore opening.

Conidia are unicellular, oblong to oval, hyaline, and measure 5-8 x 2-4 µm.

Conidia are released from the pycnidia in the form of a creamy coloured curved tendril.

Spherical, dark-brown pycnidia release unicellular, hyaline conidia through a pronounced pore opening in the form of a curved tendril.

The pycnidia of *Phoma* species often develop in compact colonies and produce spores in profusion.

Unicellular conidia distinguish *Phoma* species from the pycnidial fungi of the *Septoria* complex.

Septoria nodorum (Berk.) Berk.

Depazea nodorum Berk.
Hendersonia nodorum (Berk.) Petrak
Macrophoma hennebergii (Kühn) Berl. & Vogl.
Teleomorph: *Phaeosphaeria nodorum* (E. Müller) Hedjaroude
Leptosphaeria nodorum E. Müller

Disease

Glume blotch of wheat.

Distribution

World-wide, and especially in milder climates.

Significance

Crop production: Disease may cause significant crop losses when epidemics develop before heading.

Quarantine: Certain parts of Europe may have restrictions.

Detection technique

Freezing blotter method
(See Annex A)

Identification

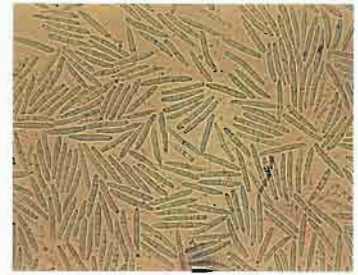
165

***Septoria nodorum* colony on wheat (x8)**

166

***Phaeosphaeria nodorum* ascocarp on wheat seed glumes (x32)**

167

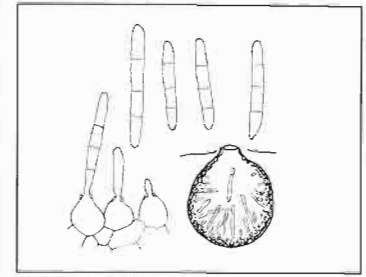


Conidia (x400)

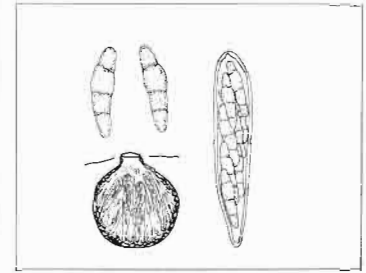
168

Ascocarp and asci (x160)

Quick clue



Septoria nodorum



Phaeosphaeria nodorum

References

Baker, C.J. 1970. Influence of environmental factors on development of symptoms on wheat seedlings grown from seed infected with *Leptosphaeria nodorum*. *Trans. Br. Mycol. Soc.* 55 (3): 443-447.

CMI. 1966. *Descriptions of Pathogenic Fungi and Bacteria No. 86. Leptosphaeria nodorum.* CAB, UK.

Richardson, M.J., and Noble, M. 1970. *Septoria* species on cereals - a note to aid their identification. *Plant Pathology* 19: 159-163.

Wiese, M.V. 1977. *Compendium of Wheat Diseases.* APS Press, USA.

Zillinsky, F.J. 1983. *Common Diseases of Small Grain Cereals.* Mexico, CIMMYT.

Colony on seed is white, olive-grey or pink with a dense cotton wool appearance. Pycnidia are found beneath mycelium on the bottom side of seed or in the mycelial mat on the blotter surface.

Occasionally, mid brown to black, spherical ascocarps are found immersed in the seed glumes and are seen if the seed is not properly cleaned.

Note: Infected wheat seeds are shrivelled.

Pycnidia are spherical, honey brown, becoming darker, 140-200 µm diameter, and rough-walled, with a pore opening which projects slightly, and measures up to 25 µm in diameter.

Conidia are hyaline, cylindrical, slender and thread-like, smooth, straight, sometimes irregularly curved, mostly 3-septate, with rounded ends, and measure 22-30 x 2-3 µm.

Ascocarps are spherical, mid brown to black, 150-200 µm diameter, with slightly projecting pore opening of 15 µm diameter.

Asci are club-shaped, cylindrical or curved, with a short stalk, 8-spored, and measure 47-65 x 8-10 µm.

Ascospores are narrow towards the ends, subhyaline to pale brown, 3-septate, narrowed at the septa, penultimate cell swollen, and 19 - 23 x 4 µm.

Septoria nodorum is identified by characteristic spherical pycnidia with hyaline, straight or slightly curved conidia having rounded ends and 3 conspicuous septa.

Conidia are shorter, thicker and straighter than any of the other *Septoria* species, and have one to three distinct septa when mature.

Pycnidia are less conspicuous than those of *S. tritici.*

Conidia are released in gelatinuous white to pink droplets or columns from the pore opening of the pycnidia when pycnidia are moistened.

Phaeosphaeria nodorum is identified by mid brown to black, spherical ascocarps with club-shaped asci comprising of 8 spindle-shaped, 3 septate ascospores.

Stenocarpella macrospora (Earle) Sutton

Diplodia macrospora Earle
Macrodiplodia macrospora (Earle) Hohnel
Macrodiplodia zeae (Schw.) Petrak & Sydow var. *macrospora* (Earle) Petrak
Stenocarpella zeae Sydow

Disease

Dry rot, diplodia ear rot, diplodia stalk rot, and diplodia macrospora leaf stripe of maize.

Distribution

World-wide.

Significance

Crop production: Minor disease in southeast USA but greater losses in Central America. Potentially higher incidence under minimum tillage.

Quarantine: Restrictions by EPPO (Europe) on list A2 (present in part of the region; of quarantine importance elsewhere) and NEPPO (Near East) on list A1 (not present in region).

Note: Mycotoxin (-diplodiol), in grain, is of minor importance.

Identification

169

Colony on maize (x8)

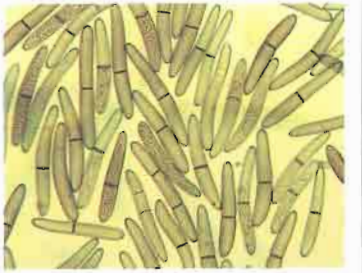
170

Colony on maize (x64)

171

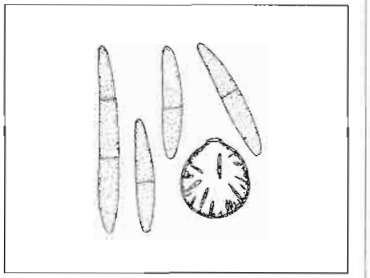
Pycnidia and conidia (x160)

172



Conidia (x400)

Quick clue



Detection technique

Freezing blotter method
(See Annex A)

References

CMI. 1966. *Descriptions of Pathogenic Fungi and Bacteria No. 83. Diplodia macrospora.* CAB, UK.

Latterell, F.M., and Rossi, A.E. 1983. *Stenocarpella macrospora* (=*Diplodia macrospora*) and *S. maydis* (=*D. maydis*) compared as pathogens of corn. *Plant Disease* 67 (7): 725-729.

Shurtleff, M.C. 1980. *Compendium of Corn Diseases.* APS Press, USA.

Colony on seed is grey with white tinge and slow growth rate. Dark brown to black pycnidia are interspersed in the mycelium and immersed in the seed surface.

Note: Infected maize seeds are grey with pycnidia on the sides and tip.

Pycnidia are dark-brown to black, spherical or almost spherical and 200-300 µm in diameter, with a multicellular wall and darker round the circular pore opening which extends out, measuring 30-40 µm in diameter.

Conidia are pale brown, smooth-walled, straight or curved, 0-3 septate, and measure 44-82 x 7-12 µm.

Stenocarpella species are distinguished by pale brown two-celled, straight to slightly curved conidia.

S. macrospora is readily distinguished from *S. maydis* which also occurs on maize by the larger, 0-3 septate conidia.

S. macrospora appears to be more generally widespread in warm humid climates than *S. maydis*.

Stenocarpella maydis (Berk.) Sutton

Diplodia maydis (Berk.) Sacc.
Sphaeria zeae Schw.
Hendersonia zeae (Schw.) Haszl.
Phaeostagonosporopsis zeae (Schw.) Woronichin

Sphaeria maydis Berk.
Diplodia zeae (Schw.) Lév.
Macrodiplodia zeae (Schw.) Petrak & Sydow
Diplodia zeae-maydis Mechtij.

Disease

Diplodia stalk rot, white ear rot, dry rot, diplodia seedling blight, and diplodiosis of maize.

Distribution

World-wide.

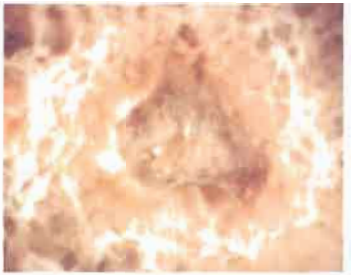
Significance

Crop production: Minor losses in USA. Currently a serious disease in South Africa. Wet weather, late in season, favours disease.

Quarantine: Restrictions for Egypt, Israel, and by EPPO (Europe) on list A2 (present in part of region; of quarantine importance elsewhere) and NEPPO (Near East) on list A1 (not present in region).

Identification

173



Colony on maize (x8)

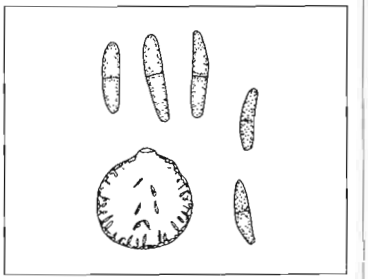
174

Pycnidia and conidia (x160)

175

Conidia (x400)

Quick clue



Detection technique

Freezing blotter method
(See Annex A)

References

CMI. 1966. *Descriptions of Pathogenic Fungi and Bacteria No. 84 Diplodia maydis.* CAB, UK.

Latterell, F.M., and Rossi, A.E. 1983. *Stenocarpella macrospora* (=*Diplodia macrospora*) and *S. maydis* (=*D. maydis*) compared as pathogens of corn. *Plant Disease* 67: 725-729.

Shurtleff, M.C. 1980. *Compendium of Corn Diseases.* APS Press, USA.

Colony on seed is grey with white tinge and slow growth rate. Black pycnidia are interspersed in the mycelium and immersed in the seed surface.

Pycnidia are spherical or flask-shaped, dark-brown to black, 150-300 µm in diameter, with a multicellular wall and darker round the circular pore opening which extends outwards measuring 40 µm in diameter.

Conidia are olive-brown, straight to slightly curved, two-celled, smooth-walled and measure 5-8 x 15-34 µm.

Stenocarpella species are distinguished by pale brown two-celled, straight to slightly curved conidia.

S. maydis is distinguished from *S. macrospora* which also occurs on maize, solely by the smaller conidia.

S. maydis appears less widespread in warm humid climates than *S. macrospora*.

Chaetomium Kunze

Disease
None.

Distribution
World-wide.

Significance
Crop production: None. Common saprophyte and secondary invader.

Quarantine: None known.

Note: Seeds of low germinating capacity are sometimes found to be heavily contaminated with *Chaetomium*.

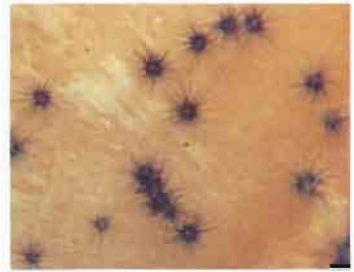
Detection technique
Freezing blotter method
(See Annex A)

Identification

176

Colony on maize (x8)

177

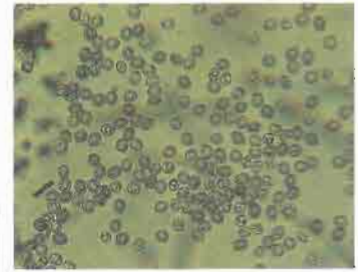


Perithecia on maize (x64)

178

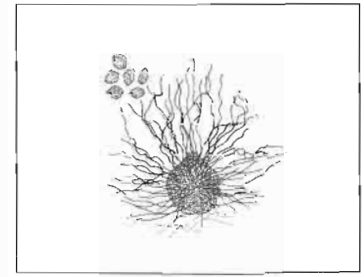
Perithecia (x160)

179



Ascospores (x400)

Quick clue



References

Malone, J.P., and Muskett, A.E. 1964. Seed-borne fungi - description of 77 fungus species. *Proc. Int. Seed Test. Assoc.* 29 (2): 179-384.

Skolko, A.J., and Groves, J.W. 1953. Notes on seed-borne fungi VII. *Chaetomium. Can. J. Bot.* 31: 779-809.

Shurtleff, M.C. 1980. *Compendium of Corn Diseases.* APS Press, USA.

von Arx, J.A., Dreyfuss, M., and Muller, E. 1984. A reevaluation of *Chaetomium* and the *Chaetomiaceae. Persoonia* 12 (2): 169-179.

Colony on seed is white with the density of mycelium varying from light to dense.

The perithecia are found on the seed surface beneath the aerial white mycelium.

Perithecia are spherical or elongate, with a pore opening, and a dark, membranous, cellular wall which is covered with conspicuous hairs of various types.

Asci are hyaline, usually club-shaped but in a few cases cylindrical, and contain eight ascospores.

Ascospores are one-celled and in most cases lemon-shaped; they are extruded through the pore opening either as a mass amongst the hairs or as a column depending on conditions.

Colonies of *Chaetomium* species can be readily recognised by the presence of perithecia with many stiff dark hairs.

Claviceps

Claviceps purpurea (Fr.) Tul. *Claviceps gigantea* Fuentes *et al.*

Disease

Ergot of wheat (*C. purpurea*) and maize (*C. gigantea*).

Distribution

C. purpurea: World-wide, but more prevalent in cool temperate climates.

C. gigantea: Mexico. Confined to high humid valleys of central Mexico

Significance

Crop production: Yield losses are less serious than losses from discounted grain quality. Maize ergot is very rarely found.

Quarantine: Restrictions by most countries for *C. purpurea*.

Note: Alkaloid chemicals in *C. purpurea* sclerotia are toxic to humans and animals.

Identification

180

Wheat spike infected with *C. purpurea*

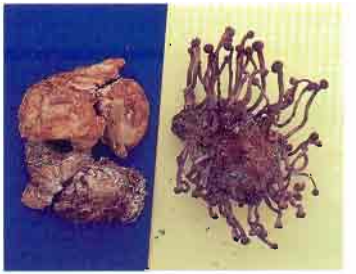
181

Maize seeds infected with *C. gigantea*

182

Stromata developing from sclerotia of *C. purpurea*

183



Stromata developing from sclerotia of *C. gigantea*

Quick clue

Detection technique

Visual examination

References

Fuentes, S.F., de Lourdes de la Isla, M., Ullstrup, A.J., and Rodriguez, A.E. 1964. *Claviceps gigantea*, a new pathogen of maize in Mexico. *Phytopathology* 54 (4): 379-381.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists*. APS Press, USA.

Ullstrup, A.J. 1973. Maize ergot: a disease with a restricted ecological niche. *PANS* 19 (3): 389-391.

Wiese, M.V. 1977. *Compendium of Wheat Diseases*. APS Press, USA.

C. purpurea sclerotia are purple-black horn-like sclerotia (ergots) with white-grey contents, which replace one or more kernels in the spike and measure 2 to 20 mm. They stick out from the glumes as spikelets mature and are up to four times larger than normal seeds.

C. gigantea sclerotia are initially white to cream-coloured, soft, sticky and hollow. Later they become hard and horny, white to brown, and often resemble a horse's tooth.

Note: Seeds of maize ear adjacent to those replaced by *C. gigantea* sclerotia are shrivelled and become coffee-coloured.

C. purpurea

Sclerotia germinate to form one or more white stalked stromata which darken with age, and reach 5-20mm in length, with flask-shaped perithecia embedded in the knob-like apex.

Perithecia are 150 x 200 μm, stand out slightly from the stroma and contain numerous hyaline club-shaped asci. Ascospores are thread-like, 3-7 septate, 8 per ascus and approximately 0.6 x 60 μm.

Conidia from honeydew stage are 2-3 x 4-6 μm, one-celled, hyaline, oval, and curved.

C. gigantea

Perithecia are arranged in stromatic heads. Ascospores are hyaline, non-septate and measure 176-186 x 1-2 μm.

Macroconidia are oval and 8-27 x 4-6 μm. Microconidia are oval and 4-7 x 2-4 μm.

Claviceps can be readily identified by visual examination. Seeds are replaced by sclerotia which are up to four times the length of normal seeds and blue-black or white to cream in colour for wheat and maize ergots respectively.

Melanospora Corda

Disease
None.

Distribution
Widespread.

Significance
Crop production: Negligible impact; common saprophyte.

Quarantine: None known.

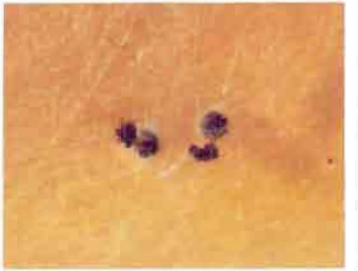
Detection technique
Freezing blotter method
(See Annex A)

Identification

184

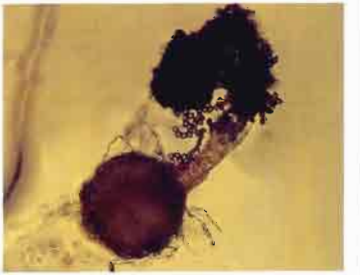
Colony on maize with *Fusarium graminearum* (x8)

185



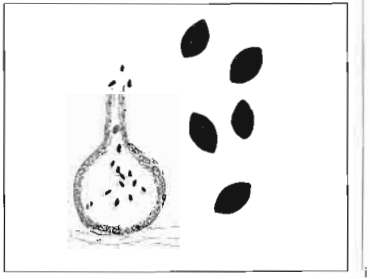
Perithecia on maize (x64)

186



Perithecia and ascospores (x160)

Quick clue



References

Malone, J.P., and Muskett, A.E. 1964. Seed-borne fungi - description of 77 fungus species. *Proc. Int. Seed Test. Assoc.* 29 (2): 179-384.

Colony on seed comprises of white aerial mycelium with golden to brown perithecia embedded in seed surface and blotter paper.

Perithecia are golden yellow to brown, spherical with long necks; and the wall of the perithecium is cellular and covered with fine cottony hyphae. The necks are 45-55 µm wide at the base, tapering slightly and terminating in a brush of blunt projections, approximately half the length of the whole neck. The perithecia measure 125-230 µm in diameter, and the overall length including neck and projections is 358-525 µm.

Asci are club-shaped, measure 65 x 35 µm and contain 8 spores arranged irregularly. The asci quickly disintegrate and are only seen in young perithecia.

Ascospores are lemon-shaped, 15-22 x 11-15 µm, dark brown to almost black. The free ascospores are forced up the neck of the perithecium and appear as a mass amongst the projections or as a column.

Golden yellow brown perithecia with long necks terminating in a brush of blunt hairs; releasing columns or masses of lemon-shaped, dark brown to black ascospores.

Sordaria Ces. & de Not.

Disease
None.

Distribution
World-wide.

Significance
Crop production: None.

Quarantine: None known.

Detection technique
Freezing blotter method
(See Annex A)

Identification

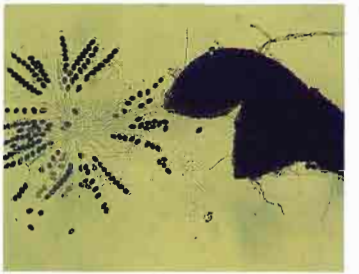
187

Colony on wheat (x8)

188

Ascocarps on wheat (x32)

189

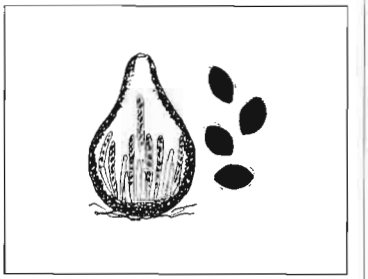


Perithecia, asci and ascospores (x100)

190

Asci and ascospores (x100)

Quick clue



References

Malone, J.P., and Muskett, A.E. 1964. Seed-borne fungi - description of 77 fungus species. *Proc. Int. Seed Test. Assoc.* 29 (2): 179-384.

Colony on seed with slow-growing white/grey mycelium and production of large, black spherical ascocarps exuding glistening black ascospores.

Perithecia are large, black, spherical, 250 µm wide, with a cylindrical neck (110 µm long and 100 µm wide), and a few hyaline hairs.

Asci are cylindrical, with a short stalk and a pore at the apex, measure 160-190 x 16-18 µm; and contain 8 ascospores.

Ascospores are glistening black, lemon-shaped, often more pointed at one end, and measure 15-22 x 11-12 µm.

Large, spherical ascocarps with glistening black lemon-shaped ascospores.

Sporisorium reilianum (Kühn) Langdon Fullerton

Sphacelotheca reiliana (Kühn) Clint.
Ustilago reiliana Kühn
Sorosporium reilianum (Kühn) McAlp.

Disease

Head smut of maize.

Distribution

World-wide.

Significance

Crop production: Losses are generally minor, but yield losses of 30 to 50% have been reported in individual fields.

Quarantine: Restrictions for Egypt and Chile.

Detection technique

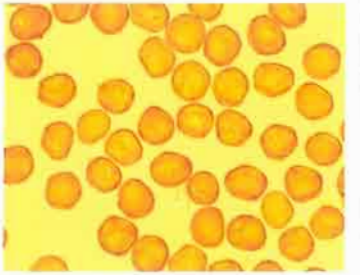
Seed wash filtration
(See Annex A)

Identification

191

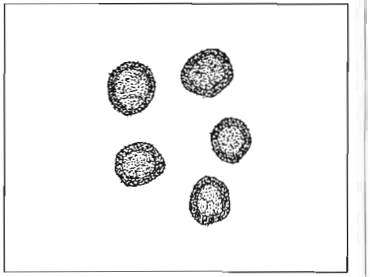
Infected maize ear (x4)

192



Teliospores (x400)

Quick clue



References

CMI. 1965. Descriptions of Pathogenic Fungi and Bacteria No. 73. *Sphacelotheca reiliana.* CAB, UK.

CMI. 1965. Descriptions of Pathogenic Fungi and Bacteria No. 74. *Sphacelotheca sorghi.* CAB, UK.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists.* APS Press, USA.

Shurtleff, M.C. 1980. *Compendium of Corn Diseases.* APS Press, USA.

Sori destroying the inflorescences, are each at first covered by an outer coat of fungal tissue which soon disintegrates into spherical or subspherical, hyaline, or pale yellowish, 'sterile cells', 5-15 µm in diameter.

Spore mass is powdery, dark brown, and quickly dispersed to expose a tangled mass of vascular strands of the host, or, rarely, a single central strand.

Teliospores are spherical to subspherical, or somewhat angled, pale yellowish to dark reddish brown to black, abundantly spiny, and 9-14 µm in diameter.

Teliospores germinate to form basidia and lateral sporidia that are small, hyaline, single-celled, and 7-15 µm in diameter.

Teliospores of *S. reilianum* can be distinguished from teliospores of *S. cruenta* and *S. sorghi* by their conspicuous spiny walls and larger diameter.

Tilletia caries (DC.) Tulasne

Uredo caries de Candolle
Tilletia tritici (Bjerk.) Wolf

Tilletia laevis Kühn

Erysibe foetida Wallr.
Ustilago foetens Berk. & Curt.
Tilletia foetens (Berk. & Curt.) Shröter
Tilletia foetens (Berk. & Curt.) Tcrel.
Tilletia foetida (Wallr.) Liro

Disease

Common bunt, hill bunt or stinking smut of wheat.

Distribution

World-wide. Occurs in most countries where wheat is grown.

Significance

Crop production: Reduction in yields and grain quality. Less frequent and usually less damaging on spring wheat than on winter wheat.

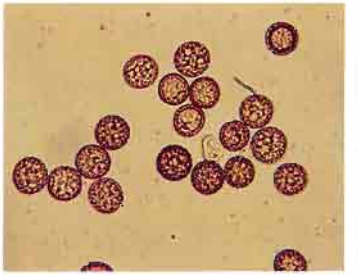
Quarantine: Restrictions for Burundi and Egypt and by IAPSC (Africa) on list A2 (present in part of region; of quarantine importance elsewhere) and NEPPO (Near East) on list A1 (not present in region).

Identification

193

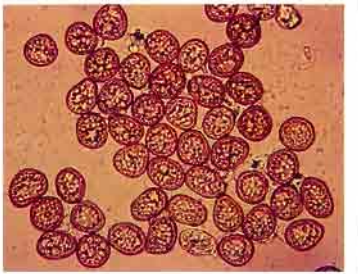
Wheat seeds infected with *T. caries* and *T. laevis* (x4)

194



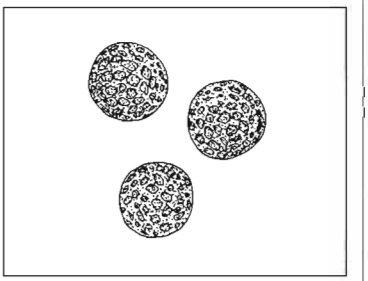
***T. caries* teliospores (x400)**

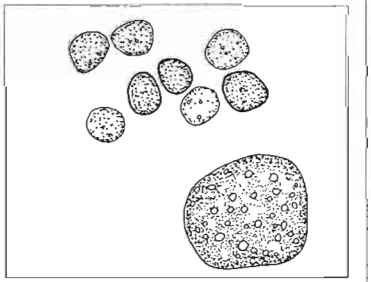
195



***T. laevis* teliospores (x400)**

Quick clue





Detection technique

Seed wash filtration
(See Annex A)

References

CMI. 1981. Descriptions of Pathogenic Fungi and Bacteria No. 719 . *Tilletia caries.* CAB, UK.

CMI. 1981. Descriptions of Pathogenic Fungi and Bacteria No. 720 . *Tilletia foetida.* CAB, UK.

Duran, R., and Fischer, G.W. 1961. *The Genus Tilletia.* Washington State University, USA.

Wiese, M.V. 1977. *Compendium of Wheat Diseases.* APS Press, USA.

Zillinsky, F.J. 1983. *Common Diseases of Small Grain Cereals.* CIMMYT, Mexico.

Sori in the ovaries filling the grain with teliospores, are partly concealed by the glumes, 4-8 mm long, and of similar diameter to uninfected grain.

Spore mass is powdery, reddish-brown to blackish, and composed of teliospores and sterile cells.

Bunt balls emit a strong fishy odour when crushed, and have a darkened appearance.

Note *T. caries* and *T. laevis* are very similar in morphological features, life cycles, disease development, and in the occurrence of physiologic races.

T. caries teliospores are without a sheath, spherical or subspherical to oval, light brown to reddish brown, 14-23 µm in diameter, and have a fine network of polygonal shapes on walls.

T. laevis teliospores are without a sheath, spherical or subspherical to oval, olive brown, 13-25 µm in diameter, and have smooth to shallowly pitted walls, often with a short mycelial fragment attached.

Sterile cells, intermixed with teliospores, are spherical, hyaline, thin-walled, smooth and 10-20 µm diameter.

T. caries and *T. laevis* differ slightly in shape of teliospores and in the texture of teliospore walls. *Tilletia caries* produces spherical or kernel-shaped bunt balls with slightly net-like walls. *T. laevis* produces spherical to more oblong shaped or irregular bunt balls with smooth walls.

T. laevis teliospores are readily distinguished from other bunts or covered smuts on wheat by the apparently smooth-walled teliospores.

Teliospores of *T. caries* resemble those of *T. controversa* but have a finer network of polygonal shapes and are not surrounded by a sheath. *T. caries* teliospores are easily distinguished from the much larger, darker and coarsely roughened *T. indica* teliospores.

Tilletia controversa Kühn

Tilletia brevifaciens Fischer

Disease

Dwarf bunt of winter wheat.

Distribution

Europe (except Spain and UK); North Africa, West Asia, North America, Argentina and Uruguay.

Significance

Crop production: Yield losses are less serious than losses from discounted grain quality for export markets. Pathogen occurs where winter wheat is subject to prolonged snow cover on unfrozen ground.

Quarantine: Restrictions by China, Turkey and Mexico; by NEPPO (Near East) on list A1 (not present in region) and by EPPO (Europe) and IAPSC (Africa) on list A2 (present in part of region; of quarantine importance elsewhere).

Identification

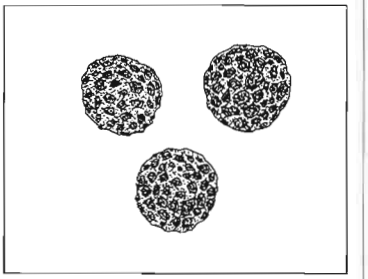
196

Infected wheat seeds (x4)

197

Teliospores (x400)

Quick clue



Detection technique

Seed wash filtration
(See Annex A)

References

CMI. 1981. Descriptions of Pathogenic Fungi and Bacteria No. 746 . *Tilletia controversa*. CAB, UK.

Duran,R., and Fischer, G.W. 1961. *The Genus Tilletia*. Washington State University, USA.

Trione, E.J. 1982. Dwarf bunt of wheat and its importance in international wheat trade. *Plant Disease* 66 (11): 1083-1088.

Wiese, M.V. 1977. *Compendium of Wheat Diseases*. APS Press, USA.

Sori in the ovaries filling the grain with spores, are on most hosts little larger than uninfected grain and therefore partly hidden by the glumes.

Spore mass is powdery, pale to dark reddish to blackish brown, and composed of spores intermixed with sterile cells.

Teliospores are spherical to subspherical, yellowish to reddish brown, 16-25 µm in diameter, and have walls with a strongly sculptured network of polygonal shapes - 1-3 µm high and 3-5 µm across. A sheath is present, and is usually inconspicuous, extending little beyond the wall sculpturing, or rarely up to 5 µm thick.

Sterile cells are spherical, hyaline, thin-walled, smooth, 10-18 µm diameter, and occasionally enclosed in a sheath.

Teliospores of *T. controversa* resemble those of *T. caries* but are more deeply sculptured and surrounded by a fragile sheath.

The teliospores are also easily distinguished from the much larger, darker and coarsely roughened *T. indica* teliospores.

Tilletia indica Mitra

Neovossia indica (Mitra) Mundkur

Disease

Karnal bunt (partial bunt) of wheat.

Distribution

India, Pakistan, Nepal, Afghanistan, Iraq, and Mexico.

Significance

Crop production: Crop yields in India and Mexico have not been significantly affected overall; however, occasionally high incidences of infected grains have caused significant reductions in quality.

Quarantine: Stringent quarantine regulations have been applied by many countries. Restrictions by Cyprus, China, Canada, Mexico, USA, and by EPPO (Europe); COSAVE (South America); IAPSC (Africa), and NEPPO (Near East) all on list A1 (not present in region).

Identification

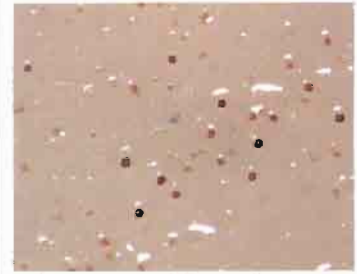
198

Infected seeds (x4)

199

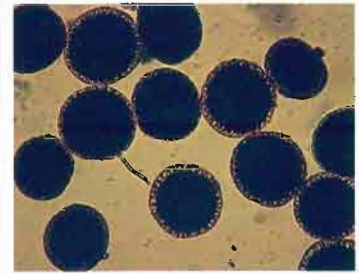
Infected seeds soaked in 0.2% NaOH (x8)

200



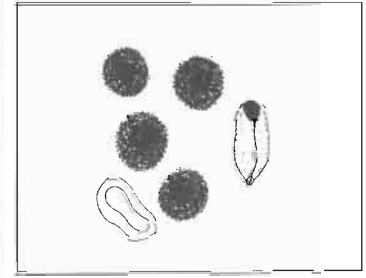
Teliospores on filter under a dissecting microscope (x64)

201



Teliospores (x400)

Quick clue



Detection technique

Seed wash filtration
(See Annex A)

References

Warham, E.J. 1986. Karnal bunt disease of wheat: A literature review. *Tropical Pest Management* 32 (3): 229-242.

CMI. 1983. Descriptions of pathogenic fungi and bacteria No. 748, *Tilletia indica*. CAB, UK.

Agarwal, V.K., and Verma, H.S. 1982. A simple technique for the detection of Karnal bunt infection in wheat seed samples. *Seed Res.* 11: 100.

Infection always begins at the embryo end of the seed and proceeds along the seed crease.

The disease is confined to the endosperm, which is eventually converted to a mass of black teliospores. However, significant portions of the pericarp, and usually of the endosperm as well, are generally left intact (hence, the name "partial bunt"). Infections occurring late in the development of the seed never progress much beyond the initial site of infection and are difficult to observe with the naked eye in dry seed samples.

However, soaking these seeds in an aqueous 0.2% NaOH solution for 24 hours produces a mild bleaching of the endosperm that makes the blackened infection points stand out in stark contrast.

Mature teliospores of *T. indica* are comparatively large, more or less spherical, and very dark (immature ones are lighter in colour). The mean diameter of mature spores, including the sheath, is usually 33-40 µm.

Wetted spores can be seen easily under a dissecting microscope; however, examination with a compound microscope is needed to confirm identification.

Partially infected grains with dark spore masses are present. Infection *always* begins at the embryo end and proceeds along the crease within the endosperm.

Large dark spores with sheaths are present. The sheath, which is generally present but may not be obvious in water alone, helps to distinguish *T. indica* spores from those of other fungal species (i.e. *Epicoccum*) and from spherical inorganic particles. Samples can be wetted with an aqueous 1-3% KOH solution to promote sheath expansion.

Ustilago maydis (DC.) Corda

Uredo maydis DC.
Uredo zeae Schw.
Ustilago zeae-mays Magnus
Ustilago zeae (Schw.) Unger

Disease

Common smut of maize.

Distribution

World-wide.

Significance

Crop production: Heavy losses can occur in susceptible varieties. Sweet corn is more susceptible than maize. Disease is favoured by dry conditions and temperatures of 26-34ºC.

Quarantine: None known.

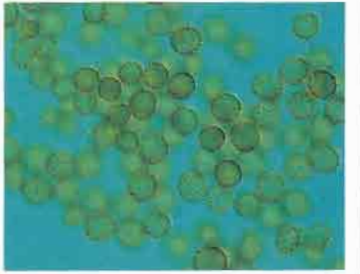
Note: Teliospores, in addition to inducing allergic disorders in man, are toxic to both man and animals.

Identification

202

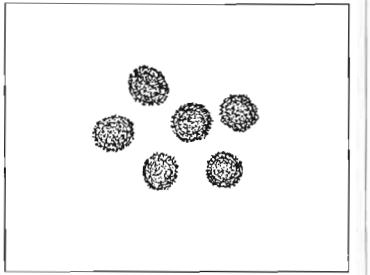
Infected maize ear

203



Teliospores (x400)

Quick clue



Detection technique

Seed wash filtration
(See Annex A)

References

CMI. 1965. Descriptions of pathogenic fungi and bacteria No. 79, *Ustilago maydis*. CAB, UK.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists*. APS Press, USA.

Shurtleff, M.C. 1980. *Compendium of Corn Diseases*. APS Press, USA.

Sori in the inflorescences, leaves, and stems are irregular swellings less than 1 cm to more than 10 cm in length; and are at first limited by a white, cream or greenish membrane of fungus and host tissue which soon ruptures.

Spore mass is powdery, and very dark brown in colour.

Teliospores are olive-brown to black, spherical or subspherical, with prominent blunt spines, and measure 8-12 µm in diameter.

Teliospores germinate by formation of a promycelium with four or more hyaline, slender, thread-like sporidia.

Very small, black, spherical, teliospores measuring 8-12 µm in diameter.

Ustilago nuda (Jensen) Rostrup

Uredo carbo DC.
Ustilago segetum (Pers.) Ditmar
Ustilago tritici (Pers.) Rostrup

Disease

Loose smut of wheat and barley.

Distribution

World-wide.

Significance

Crop production: Losses are normally less than 1% of the ears of the crop but the introduction of a susceptible cultivar as in the case of the British barley crop in 1969 may increase infection as much as 20%. Spring crops are mainly affected.

Quarantine: Restrictions for Mexico and other countries.

Detection technique

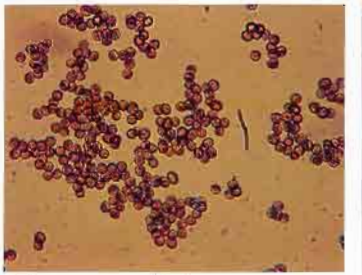
Embryo staining technique

Identification

204

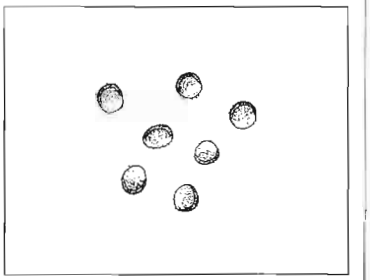
Infected barley spike

205



Teliospores (x400)

Quick clue



References

CMI. 1970. Descriptions of pathogenic fungi and bacteria No. 280, *Ustilago nuda*. CAB, UK.

Malone, J.P., and Muskett, A.E. 1964. Seed-borne fungi - description of 77 fungus species. *Proc. Int. Seed Test. Assoc.* 29 (2): 179-384.

Zillinsky, F.J. 1983. *Common Diseases of Small Grain Cereals*. CIMMYT, Mexico.

Sori in spikelets replace the ovaries, and are temporarily protected by a thin membrane but become dispersed, at maturity; leaving the bare rachis.

Spore mass is powdery olive brown in colour.

Note: Infected seed may be reduced in size and lighter than healthy seed.

Teliospores are spherical to subspherical or sometimes irregular, pale yellow brown, and lighter in colour on one side and measure 5-9 µm in diameter. They possess short spines on their surface which are more noticeable on the paler side.

Germinating teliospores produce a four-celled promycelium, but no sporidia.

Very small teliospores, measuring 5-10 µm in diameter.

U. nuda is differentiated from the black loose smut pathogen *U. avenae* by the absence of sporidia production on potato dextrose agar.

The short spines on spore walls of *U. nuda* and *U. avenae* readily distinguish these species from *U. hordei* which has smooth walls.

Rhizopus Ehrenb.

Disease
Rhizopus ear rot of maize.

Distribution
World-wide.

Significance
Crop production: Minor losses of no significant importance.
Can be important in storage rot complex under elevated moisture and temperature conditions.

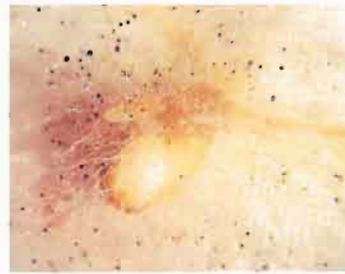
Quarantine: None known.

Note: *Rhizopus* growth, originating from seeds, commonly occurs in laboratory germination tests, but does not seem to affect results.

Detection technique
Freezing blotter method
(See Annex A)

Identification

206

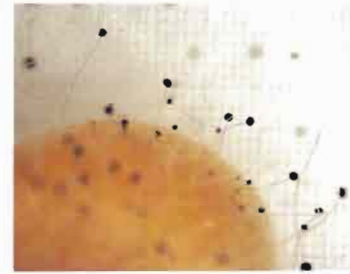


Colony on wheat (x8)

207

Colony on maize (x8)

208

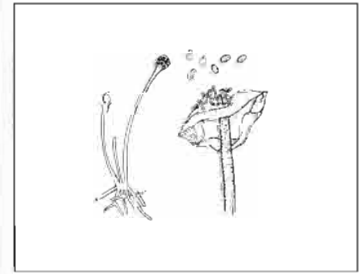


Sporangiophores (x20)

209

Sporangiophores and spores (x100)

Quick clue



References

CMI. 1977. Descriptions of Pathogenic Fungi and Bacteria No. 524, *Rhizopus stolonifer*. CAB, UK.

CMI. 1977. Descriptions of Pathogenic Fungi and Bacteria No. 525, *Rhizopus oryzae*. CAB, UK.

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists*. APS Press, USA.

Malone, J.P., and Muskett, A.E. 1964. Seed-borne fungi - description of 77 fungus species. *Proc. Int. Seed Test. Assoc.* 29 (2): 179-384.

Colony on the seed spreads rapidly by means of stolons with abundant loose grey mycelium.

Stolons produce numerous brown sporangiophores, and rhizoids.

Note: The fungus is so common on maize seeds, that tests for other pathogens often employ precautionary measures to avoid growth of *Rhizopus*, for example by surface sterilisation of seeds with sodium hypochlorite.

Stolons are hyaline becoming brown towards nodes, near which a septum may occur.

Rhizoids are short, brown and sometimes absent.

Sporangiophores arising singly or in small groups from nodes on the stolons, are brown, smooth or finely roughened, non-septate, 1000-3500 µm long and up to 34 µm wide.

Sporangia are spherical, initially white but later black, and 100-350 µm in diameter with numerous spores.

Columellae are light brown, sub-spherical, 63-224 x 70-140 µm, and umbrella-shaped when dehisced.

Sporangiospores are yellowish to dilute brown, spherical or oval, longitudinally striped, and measure 5-8 x 20-26 µm.

Dark, spherical sporangia can readily be seen under a low magnification(i.e. dissecting microscope), enabling identification of *Rhizopus* without removal of the lid.

Often referred to as pin mould as sporangia resemble black pin heads and are widely interspersed in cotton wool-like mycelium.

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Annex A
Seed Health Tests

Seeds carry a microflora which varies with the host species. This is especially true for the more deeply seated microfloras, whilst on the surface many accidental guests may be carried as well. The seed-borne microflora can be identified through the use of seed health tests.

Seed health tests include:

1. Examination of seeds externally or internally, macro- or micro-scopically, for the presence of pathogens (visual examination, filter wash test, embryo test).
2. Incubating seeds on agar or moist blotter paper and identifying the emergent organisms (agar plate test, blotter test, and freezing method).
3. Germinating seeds and growing the seedlings in conditions known to encourage the production of diagnostic symptoms (seedling tests).
4. DNA probes for certain pathogens (e.g. Stewart's Wilt of maize).
5. ELISA tests (bacteria and viruses).

The first three techniques are routinely used for seed-borne fungi and are described below.

Seed Examination

Some seed-borne pathogens are identified by visual examination of the seed; for example, in wheat when no normal seed is produced and the seed is replaced by sori of spores of bunts and smuts, ergot, sclerotia or nematode galls. These pathogens can then be confirmed by microscopic examination of the structures. Often spores of these pathogens are transported on the surface of other seeds and their presence can only be confirmed by the use of the filter wash test.

If seeds are severely infected by some organisms they may be discoloured; for example, maize seeds infected with *Nigrospora* have white streaks with black spore masses near the tips, and wheat seeds severely infected with *Fusarium* spp. are shrivelled and have a pinkish colour.

Loose smut of wheat and barley infect the embryo and can be detected by staining and microscopic examination of the embryo using the embryo test.

Visual examination

Whole seeds are carefully examined for the presence of sori, sclerotia, galls or severe discolouration. A low power illuminated magnifying glass is recommended.

Filter wash test

Seeds are agitated with water and the wash water passed through filters. The filters are then soaked in dilute potassium hydroxide and examined under a microscope, principally for the presence of teliospores or oospores, but spores of other pathogens as well.

Embryo test

Seeds are soaked in trypan blue and sodium hydroxide to stain the infected embryos and to separate them from the endosperm and pericarp. After embryos are

separated from the chaff and other debris, they are mounted in lactophenol and examined under a steroscopic microscope for the presence of infected embryos.

Incubation tests

In the agar plate test, blotter test and freezing method, the seeds are incubated for a certain period on, or in, a particular medium under specific environmental conditions, in order to allow the pathogens on the seed to express their presence: this can be by the appearance either of symptoms of the disease, or signs of growth of the pathogen itself.

The different fungi are identified by features such as form, length and arrangement of conidiophores, the form, size, septation, colour, chain formation, etc., of conidia, and their arrangement on the conidiophores, appearance of spore masses, characters of mycelium, density of colonies, etc.

The major factors promoting growth, fructification and development of symptoms of pathogens are temperature, humidity, light and period of incubation. These are discussed below with reference to standardization in the following protocols as prescribed by the International Seed Testing Association (ISTA).

Temperature

Incubation tests are greatly influenced by temperature, which affects germination, growth, and reproduction of organisms. The temperature response curve, defining the minimum, optimum and maximum temperature, is specific for each of the different life processes of an organism, although some curves may more or less coincide.

The curve for mycelial growth may differ from the curves for sporulation, germination and infection. The temperature response curve for development of symptoms in one host may differ significantly from the curve for infection of another host by the same pathogen. Temperature affects the morphology of fungi and conditions for normal typical development are sometimes confined to a sharply defined range. Therefore, the incubation temperature for culture tests depends largely on the pathogen to be detected as well as the testing procedure to be applied. A test based on mycelial growth may require temperatures different from those for seedling growth and development of symptoms.

In general practice, however, seeds are incubated on substrates at a constant temperature. For wheat and maize, the ISTA prescribes 20°C and 20-30°C, respectively, which favours growth of a large range of pathogens.

Humidity

In the blotter test, the main problem is to keep the blotters adequately moist throughout the period of incubation, preferably under such conditions that further supply of water during this period is not needed. An increase in the amount of water and number of layers of blotters per Petri dish increases the amount of water available, and a reduction of the number of seeds per container, especially of larger seeds, such as those of cereals, decreases total consumption of water for germination. It is important that blotters be adequately moist, but too much water is detrimental to the test because it favours bacterial growth.

For the agar plate test, 2% nutrient agar is usually used. The water content of this medium is more than sufficient for the duration of the test.

Light

Light, temperature and humidity are known to influence profoundly the reproduction of fungi. General growth characteristics, extent of sporulation, spore morphology and pigmentation are also markedly influenced by light.

In general, cool white fluorescent lamps are used but the use of fluorescent black light lamps which emit radiation from near ultraviolet light to visible light (320-420 nm) could be advantageous. They give a much higher proportion of near ultraviolet at 360 nm, which appears to be most suitable for routine seed health testing. This wavelength induces sporulation of a broad range of organisms without adverse effects.

Some light-sensitive fungi sporulate when exposed continuously to near ultra-violet light, whilst others require a subsequent period of darkness to complete spore production. In the last-mentioned group, termed diurnal sporulators, sporulation is divided into two distinct phases — an 'inductive phase', resulting in the formation of conidiophores, and a second 'terminal phase' or 'conidial phase' which results in the formation of conidia. To induce sporulation of these diurnal sporulators, near ultraviolet light-darkness cycles of 12/12 h are adopted.

Length of incubation

The length of the incubation period for any organism under test is prescribed by the rate of growth of the organism. However, in most routine seed-testing, efforts are made to obtain easily readable results and maximum numbers. The length of incubation depends on other factors influencing the rate of development of the organisms involved. Temperature is particularly important. At incubation temperatures lower than the standard 20°C for wheat, in which 10 days incubation is usually required for adequate detection of most fungi, the length of incubation must be increased accordingly to obtain approximately the same development of colonies.

Standard incubation procedure

Therefore, the standard incubation procedure is:

- Number of seeds: 400
- Moisture: substrate moist enough to supply optimal moisture to the seeds thoughout the test
- Substrate: filter/blotting paper or agar
- Temperature: maize 20-30°C
  wheat 20°C
- Light: near ultraviolet light-darkness cycles of 12/12 h
- Duration: maize 10 days
  wheat 10 days

Seedling tests

Seeds are germinated according to the standard seed germination tests (given in Annex B) and the seedlings are examined for diagnostic symptoms; for example, wheat seedlings infected with *Septoria nodorum* show a wide range of symptoms and such seedlings, although not killed or severely damaged, are severely stunted or distorted and bear brown lesions. In some cases, the incubation temperature of the germination test may be altered to favour development of the pathogen; for example, infection of seedlings with *Septoria nodorum* occurs more readily in the range of 10-20°C.

Working sample

The working sample for each of the seed health tests should be carefully drawn using an approved seed sample divider.

Uses of seed health tests

Routine seed health investigations of seeds may be used for several purposes:

1. To assess the incidence of a seed-borne pathogen that may affect seed quality.
2. To detect organisms of quarantine concern.
3. To determine seed quality in terms of germinability and/or vigour.
4. To determine if pesticide treatment of the seed is necessary.

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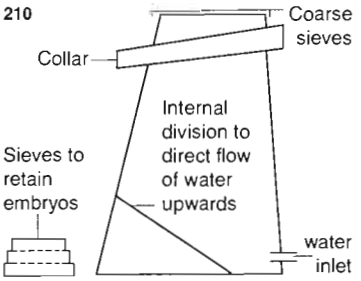
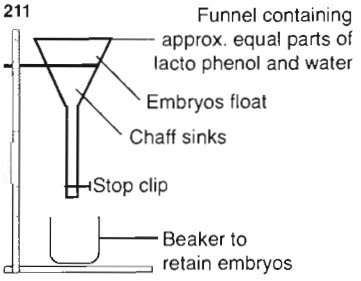
Seed Health Test

Embryo test to detect loose smut of wheat and barley

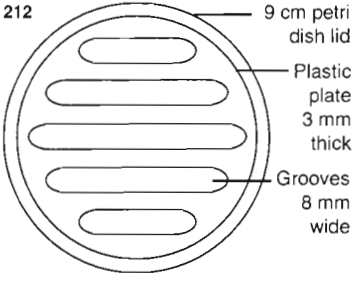
Crop: Wheat
Sample: 100-120 g containing 2000-4000 seeds



Fenwick can for embryo extraction



Separation of embryos and chaff



Embryo examination plate

Procedure:

1) Soak seeds in 1 litre of a 5% solution of fresh, aqueous NaOH containing 0.2 g trypan blue, at approximately 20°C for 22-24 hours. A weaker solution of NaOH or a lower temperature makes extraction difficult.

2) After soaking, wash seeds in warm water to separate the embryos which appear through the ruptured pericarps. A Fenwick can of the type used to wash eelworm cysts from soil samples can be used. A basal hot water inlet helps to separate the embryos and carries them to the top of the can. If necessary, agitate further by stirring. Catch the detached embryos flowing over the lip of the Fenwick can in a sieve of 1 mm mesh, and use additional sieves of larger mesh to collect pieces of endosperm and chaff.

3) Separate the embryos from the chaff and other debris in a funnel containing approximately equal parts of lactophenol (one third each of glycerol, phenol and lactic acid) and water.

When the specific gravity has been correctly adjusted, the embryos float and the chaff sinks and can be run off through a rubber tube with a stop clip. Repeat the process several times until a reasonably clean sample has been obtained.

4) Clear the embryos in fresh water-free lactophenol maintained at boiling point for approximately 30 seconds.

5) Totally immerse the embryos in fresh glycerol and arrange in rows for easier examination and counting. A plate 3 mm thick with grooves, placed inside a plastic Petri dish lid, can be used.

Evaluation:

Examine each embryo carefully under a stereoscopic microscope x18-25, with substage illumination. Count the infected embryos and the total number of embryos examined. Mycelium of *Ustilago nuda* is approximately 3 µm thick, golden brown in colour and not easily visible without the addition of a stain. When trypan blue is added to the NaOH, mycelium appears blue in infected embryos. Infection may vary from a few strands of short hyphae to complete invasion of the scutellum tissues. Staining can be variable with the mycelium in embryos that are difficult to extract only lightly stained. Occasionally fungi other than *Ustilago nuda* occur in the scutellum but are usually darker in colour and quite distinct. When cell walls become discoloured, they may be confused with mycelium of *U. nuda*, but this can be checked by examination at x50, magnification or higher.

Result:

The percentage of loose smut infection is determined by the number of infected embryos and the number of embryos examined and **not** the number of seeds soaked.

Notes:

a) Wheat embryos are more easily damaged during the extraction procedure than barley embryos, and care must be taken to prevent excessive scutellum damage.

b) The embryos are examined in fresh glycerol to avoid the unpleasant and potentially hazardous fumes of lactophenol.

References

Rennie, W.J., and Seaton, R.D. 1975. Loose smut of barley. The embryo test as a means of assessing loose smut infection of seed stocks. *Seed Science and Technology* 3: 697-709.

Rennie, W.J. 1982. ISTA *Handbook on Seed Health Testing - Working Sheet No. 25 (2. Ed.) - Loose Smut of Barley*. ISTA, Switzerland.

Rennie, W.J. 1982. ISTA *Handbook on Seed Health Testing - Working Sheet No. 48 - Loose Smut of Wheat*. ISTA, Switzerland.

Seed Health Test

Filter wash test to detect smut and downy mildew spores

Crop: Wheat
Sample: x replicates of 25-50 seeds

Procedure:

1) Place seeds in 100 ml of distilled water with one drop of Tween 20 in a 250 ml flask.

2) Agitate flasks on a shaker for 30 minutes.

3) Rinse seed three times with distilled water and pass wash water through a Whatman #1 filter paper in a Buchner funnel.

4) Once all the wash water has been filtered, store filters in individual Petri dishes until evaluation.

Evaluation:

Filter papers are soaked with a 1% solution of potassium hydroxide and examined under a microscope for the presence of teliospores or oospores.

References

Unpublished. Modifications of this technique are used by the CIMMYT Seed Health Unit.

Seed Health Test

Blotter test

Crop: Wheat
Sample: 400 seeds (4 replicates of 100 seeds)

Pretreatment (optional):

Soak seeds for 3 minutes in a 10% solution of commercial sodium hypochlorite laundry bleach and rinse (optional) with distilled water.

Procedure:

1) Place seeds evenly spaced on 2-4 layers of moist filter or blotter paper inside a transparent plastic box.

2) Seal box with parafilm.

3) Incubate box at 20°C for 10 days (with 12 h cool white or near UV light and 12 h dark per day).

Evaluation:

Observe fungal colonies with dissecting and compound light microscopes.

Applications:

Provides excellent conditions for development of mycelium growth of many hyphomycetes, for conidial sporulation of many imperfect fungi, and for development of signs of infection produced by pathogenic forms of these fungi in seedlings germinating during incubation.

Limitations:

a) Fungi which produce little or no mycelium or do not sporulate profusely under conditions of the blotter test may readily be overgrown by more vigorous fungi.

b) Pathogenic bacteria are very rarely observed in blotter tests.

c) Many important seed-borne obligate pathogens which do not produce vegetative structures under test conditions are not revealed in the blotter test (e.g. smuts).

References

Neergaard, P. 1977. *Seed Pathology*, Vol. I and II. John Wiley & Sons, New York.

Seed Health Test

Freezing blotter test

Crop: Wheat
Sample: 400 seeds (5 replicates of 80 seeds)

Pre-treatment (optional):

Soak seeds for 3 minutes in a 10% solution of commercial sodium hypochlorite laundry bleach and rinse (optional) with distilled water.

Procedure:

1) Place seeds evenly spaced on 2 - 4 layers of moist filter or blotter paper in a transparent plastic box.

2) Seal box with parafilm.

3) Incubate box at 20°C for 2 days (with 12 h cool white or near UV light and 12 h dark per day).

4) Transfer box to freezer at -15°C to -20°C for 1 day.

5) Replace box in incubator at 20°C for 11 days (with 12 h cool white or near UV light and 12 h dark per day).

Evaluation:

Observe fungal colonies with dissecting and compound light microscopes.

Application:

Used as a modification of the blotter test with the freezing period killing the seeds and thus providing a substrate for development of fungi uninhibited by plant resistance.

Advantages:

a) Percentages of infection obtained are related to those obtained in agar plate tests; the dead seed provides a food base as an agar medium without host resistance operating as in the blotter test.

b) Fewer materials and less labour needed than for the agar plate test.

c) Sporulation of certain pathogens (e.g. *Septoria* spp.), often overgrown in agar plate tests, may be favoured by this procedure.

d) Discrete colonies can be observed.

Limitations:

a) Unlike the standard blotter test, seedling infection cannot be observed.

b) The freezing blotter test favours development of saprophytes (e.g. *Alternaria tenuis, Penicillium expansum, Rhizopus* spp., *Mucor* spp. and *Aspergillus* spp.), which may obscure recording of pathogens. Such contamination may be controlled by pre-treatment of seed.

References

Neergaard, P. 1977. *Seed Pathology*, Vol I and II. John Wiley & Sons, New York.

Seed Health Test

Agar plate test

Crop: Wheat
Sample: x replicates of 10 seeds

Agar:

The type of agar used will depend on the species to be identified. The most common are malt extract and potato dextrose agars.

Pre-treatment (optional):

Soak seeds for 3 minutes in a 10% solution of commercial sodium hypochlorite laundry bleach and rinse (optional) with distilled water.

Procedure:

1) Place 10 seeds evenly spaced in each agar plate.

2) Seal Petri dishes with parafilm.

3) Incubate Petri dishes at 20°C for 5 - 8 days (with 12 h cool white or near UV light and 12 h dark per day).

Evaluation:

Observe fungal colonies under a binocular microscrope.

Application:

The agar plate test is applicable to those kinds of seeds in which saprophytic species will not inhibit the rapid identification of the pathogens. However, if present, this difficulty may be overcome by pre-treatment.

Advantages:

The use of selective or semi-selective media allows differentiation of organisms of interest.

Limitations:

a) Slow growing fungi may be suppressed by vigorously growing fungi.

b) Expensive test because of materials required.

References

Neergaard, P. 1977. *Seed Pathology*, Vol I and II. John Wiley & Sons, New York.

Seed Health Test

Semi-selective agar test for *Septoria nodorum*

Crop: Wheat
Sample: x replicates of 5 - 10 seeds

Pre-treatment (optional):

Soak seed for 3 minutes in 10% solution of commercial sodium hypochlorite laundry bleach and rinse (optional) with distilled water.

Procedure:

1) Place seeds evenly spaced on Oxgall media in Petri dishes (not exceeding 10 seeds/dish) and seal with parafilm.

2) Incubate Petri dishes at 20°C for 6 - 8 days.

Evaluation:

1) After 2 days, *S. nodorum* colonies produce a metabolite which fluoresces under near ultraviolet light.

Seed Health Test

Semi-selective agar test for *Fusarium* spp.

Crop: Wheat
Sample: x replicates of 5 - 10 seeds

Pre-treatment (optional):

Soak seed for 3 minutes in a 10% solution of commercial sodium hypochlorite laundry bleach and rinse (optional) with distilled water.

Procedure:

1) Place seeds evenly spaced on SNA agar in Petri dishes (not exceeding 10 seeds/dish) and seal with parafilm.

2) Incubate Petri dishes at 17°C for 10 - 14 days (with 12 h cool white or near UV light and 12 h dark per day).

Evaluation:

Observation of conidiophores and conidia with a compound light microscope.

Seed Health Test

Filter wash test to detect smut and downy mildew spores

Crop: Maize
Sample: x replicates of 40 seeds

Procedure:

1) Place seeds in 100 ml of distilled water with one drop of Tween 20 in a 250 ml flask.

2) Agitate flasks on a shaker for 30 minutes.

3) Rinse seed three times with distilled water and pass the wash water through a Whatman #1 filter paper in a Buchner funnel.

4) Once all the wash water has been filtered, store filters in individual plastic Petri dishes until evaluation.

Evaluation:

Filter papers are soaked in a 1% solution of potassium hydroxide and examined under a microscope for the presence of teliospores or oospores.

References

Unpublished. Modifications of this technique are used by the CIMMYT Seed Health Unit.

Seed Health Test

Blotter test

Crop: Maize
Sample: 400 seeds (4 replicates of 100 seeds)

Pretreatment (optional):

Soak seeds for 3 minutes in a 10% solution of commercial sodium hypochlorite laundry bleach and rinse (optional) with distilled water.

Procedure:

1) Place seeds evenly spaced on 2-4 layers of moist filter or blotter paper inside a transparent plastic box.

2) Seal box with parafilm.

3) Incubate box at 25°C for 10 days with 12 h cool white or near UV light and 12 h dark per day.

Evaluation:

Observe fungal colonies with dissecting and compound light microscopes.

Applications:

Provides excellent conditions for development of mycelium growth of many hyphomycetes, for conidial sporulation of many imperfect fungi, and for development of signs of infection produced by pathogenic forms of these fungi in seedlings germinating during incubation.

Limitations:

a) Fungi which produce little or no mycelium or do not sporulate profusely under conditions of the blotter test may readily be overgrown by more vigorous fungi.

b) Pathogenic bacteria are very rarely observed in blotter tests.

c) Many important seed-borne obligate pathogens which do not produce vegetative structures under test conditions are not revealed in the blotter test (e.g. smuts).

References

Neergaard, P. 1977. *Seed Pathology*, Vol. I and II. John Wiley & Sons, New York.

Seed Health Test

Freezing blotter test

Crop: Maize
Sample: 400 seeds (8 replicates of 40 seeds)

Pre-treatment (optional):

Soak seeds for 3 minutes in a 10% solution of commercial sodium hypochlorite laundry bleach and rinse (optional) with distilled water.

Procedure:

1) Place seeds evenly spaced on 2 - 4 layers of moist filter or blotter paper in a transparent plastic box.

2) Seal box with parafilm.

3) Incubate box at 25°C for 2 days (with 12 h cool white or near UV light and 12 h dark per day).

4) Transfer box to freezer at -15°C to -20°C for 1 day.

5) Replace box in incubator at 25°C for 11 days (with 12 h cool white or near UV light and 12 h dark per day).

Evaluation:

Observe fungal colonies with dissecting and compound light microscopes.

Application:

Used as a modification of the blotter test with the freezing period killing the seeds and thus providing a substrate for development of fungi uninhibited by plant resistance.

Advantages:

a) Percentages of infection obtained are related to those obtained in agar plate tests; the dead seed provides a food base as an agar medium without host resistance operating as in the blotter test.

b) Fewer materials and less labour needed than for agar plate test.

c) Sporulation of certain pathogens (e.g. *Septoria* spp.), often overgrown in the agar plate test, may be favoured by this procedure.

d) Discrete colonies can be observed.

Limitations:

a) Unlike the standard blotter test, seedling infection cannot be observed.

b) The freezing blotter test favours development of saprophytes (e.g. *Alternaria tenuis, Penicillium expansum, Rhizopus* spp., *Mucor* spp. and *Aspergillus* spp.), which may obscure recording of pathogens. Such contamination may be controlled by pre-treatment of seed.

References

Neergaard, P. 1977. *Seed Pathology*, Vol I and II. John Wiley & Sons, New York.

Seed Health Test

Agar plate test

Crop: Maize
Sample: x replicates of 5 seeds

Agar:

The type of agar used will depend on the species to be identified. The most common are malt extract and potato dextrose agars.

Pre-treatment (optional):

Soak seeds for 3 minutes in a 10% solution of commercial sodium hypochlorite laundry bleach and rinse (optional) with distilled water.

Procedure:

1) Place 5 seeds evenly spaced in each agar plate.

2) Seal Petri dishes with parafilm.

3) Incubate Petri dishes at 25°C for 5 - 8 days (with 12 h cool white or near UV light and 12 h dark per day).

Evaluation:

Observe fungal colonies under a binocular microscrope.

Application:

The agar plate test is applicable to those kinds of seeds in which saprophytic species will not inhibit the rapid identification of the pathogens. However, if present, this difficulty may be overcome by pre-treatment.

Advantages:

The use of selective or semi-selective media allows differentiation of organisms of interest.

Limitations:

a) Slow growing fungi may be suppressed by vigorously growing fungi.

b) Expensive test because of materials required.

References

Neergaard, P. 1977. *Seed Pathology*, Vol I and II. John Wiley & Sons, New York.

Seed Health Test

Stewart's wilt test

Crop: Maize
Sample: x replicates of 50 seeds

Procedure:

1) Place seeds evenly spaced on 2-4 layers of moist filter or blotter paper in a plastic tray and cover with 1 cm of unsterile soil.

2) Incubate tray at 25°C for 10 days.

Evaluation:

Observe seedlings for water-soaked lesions with wavy margins.

References

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists.* APS Press, Minnesota, USA.

Seed Health Test

Downy mildew test

Crop: Maize
Sample: x replicates of 40 seeds

Procedure:

1) Soak seeds for 48 hours in 5% sodium hydroxide and 0.05% aniline blue solution.

Evaluation:

Mash soaked seeds and examine pulp with binocular microscope for presence of blue stained oospores.

References

McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists.* APS Press, Minnesota, USA.

Media

Cornmeal agar

Ingredients:

Cornmeal	30 g
Agar	20 g
Distilled water	1 litre

Procedure:

If commercial cornmeal agar is not available, the medium may be prepared as follows:

1) Place the cornmeal and water in a saucepan. (If meal is not available, break up 30 - 35 g of maize grains and grind fairly fine or mill in a coffee grinder.)

2) Heat over a double saucepan (water bath) until boiling, stir and boil for 1 h.

3) Filter through muslin, add agar and boil till dissolved.

4) Autoclave at 120°C and 15 p.s.i. (1.05 kg/cm^2) for 20 minutes.

5) Allow agar to cool to 40-45°C.

6) Pour agar into Petri dishes and allow to cool completely prior to use and/or sealing with parafilm.

Note:

For *Phytophthora* and *Pythium* spp. and similar sensitive species, 0.5 g wheat germ oil may be added.

Media

Oxgall agar

Ingredients:

A) Base medium

Distilled water	1000 ml
Dextrose	10 g
Peptone	10 g
Oxgall	15 g
Agar	20 g

B) **Non-autoclavable** antibiotic components (optional)

Streptomycin	0.10 g
Chlorotetracyclin	0.05 g

Procedure:

1) Autoclave ingredients A) at 120 C and 15 p.s.i. (1.05 kg/cm^2) for 20 minutes and allow to cool to 40-45°C.

2) Add streptomycin (0.1 g/1000 ml) and chlorotetracyclin (0.05 g/1000 ml) and gently agitate flask to mix antibiotics.

3) Pour agar into Petri dishes and allow to cool completely prior to use and/or sealing with parafilm.

Media

SNA

Ingredients:

Distilled water	1000.0 ml
KH_2PO_4	1.0 g
KNO_3	1.0 g
$MgSO_4.7H_2O$	0.5 g
KCL	0.5 g
Glucose	0.2 g
Sucrose	0.2 g
Agar	15.0 g

Procedure:

1) Autoclave above ingredients at 120°C and 15 p.s.i. (1.05 kg/cm^2) for 20 minutes and allow to cool to 40-45°C.

2) Pour agar into Petri dishes and allow to cool completely prior to use and/or sealing with parafilm.

References

Nirenberg, H.I. 1981. A simplified method for identifying *Fusarium* spp. occurring on wheat. *Canadian Journal of Botany* 59: 1599-1609.

Identification Procedures

Scotch-tape Method

Procedure:

1) Cut a small section of sellotape approximately 4 cm long.

2) Gently hold the sellotape at each end between the thumb and forefinger with the sticky side pointing downwards in a U shape and the least amount of sellotape in contact with the fingers as possible.

3) Gently place the bottom of the U onto the surface of a colony culture so that the sticky side picks up some mycelium and conidia from the colony. Contact with the colony should be very light so as to only pick up a very small amount of mycelium.

4) Place the piece of sellotape on top of a drop of water on a slide without touching the middle section of the sellotape.

5) Place a coverslip on top of the sellotape.

Evaluation:

Observe slide under microscope.

Application:

Used to assist identification of different organisms by preserving attachment of conidia to conidiophores. It is particularly useful for those organisms in which the conidia readily detach themselves from the conidiophore (e.g *Cladosporium* spp.) or those in which chains of conidia readily break up (e.g. *Fusarium moniliforme*) under normal procedures for slide preparation.

Annex B
Seed Viability, Germination and Vigour Tests

Seed-borne diseases are one of a number of factors which affect deterioration of seed, including climatic conditions before harvest, seed maturity, mechanical damage, storage environment (temperature and humidity), insects and genetics. Seed deterioration can be quantitatively measured by evaluating a sample of the seed lot using three different criteria:

a) **Seed viability** - the capacity of the seed to live, grow, and develop;

b) **Seed germination** - the capacity of seeds to develop normal seedlings in optimum conditions; and

c) **Seed vigour** - the properties of the seed which determine the potential for uniform and rapid emergence and the development of normal seedlings in a wide range of field conditions.

Seed viability tests give results quickly, usually within two days, but can be quite labour intensive. The results often do not correlate with field emergence and are therefore not routinely used. Seed germination tests remain the principal and internationally accepted criterion for measuring seed viability. A germination test result less than an acceptable standard (for example 90% for wheat) usually indicates seed deterioration, and therefore by itself is a strong indicator that seed lot performance is likely to be poor.

However, even in high germinating seed lots, germination tests alone may not provide enough information as to potential field performance, and it is in these circumstances that the vigour status of the seed lot becomes important and vigour testing is necessary.

When seeds are sown in optimum conditions (e.g. seed bed and environmental factors), field emergence will correlate well with germination and seed lot vigour is not important. However, optimum field conditions are rarely encountered in practice, and environmental stresses (for example, soil-borne diseases, low or high soil temperature, excess or low soil moisture) will lead to varying field performance depending on the vigour status of the seed lot. Differences in field performance include varying emergence rates, and uniformity of crop growth. High vigour seed lots will perform better under environmentally stressed seed bed conditions than low vigour seed lots, even though the laboratory germination of the seed lots may not differ.

The storage potential of seed lots is related to their vigour status on entering storage. High vigour seed lots will be better able to withstand poor storage conditions. However, even with storage under controlled conditions (i.e. low temperature and low relative humidity), field performance can still depend on the vigour status of the seed lot.

Seed lots being transported within a country or exported to other countries may encounter environmental hazards (e.g. severe fluctuations in temperature and relative humidity) while in transit. High vigour seed lots are more likely to be able to withstand these stresses than low vigour seed lots.

The protocols for seed viability, seed germination, and seed vigour tests used for wheat and maize are described below (with standardization of incubation periods, etc., according to ISTA rules for seed testing).

Tetrazolium seed viability test

In living cells, dehydrogenase enzymes reduce colourless tetrazolium chloride salt to form the water insoluble red compound formazan. The red-coloured living parts of the seeds can then be distinguished from the colourless dead parts.

Seed germination tests

Seed germination tests involve sowing seeds on a uniform substrate (rolled paper towels, sterilised sand, etc.) and incubating the test at an optimum temperature for germination of the seeds. After the prescribed period of incubation, the numbers of normal seedlings, abnormal seedlings, and ungerminated seeds are determined.

The standard incubation procedures for seed germination tests for wheat and maize are as follows:

- Number of seeds: 400
- Moisture: substrate moist enough to supply optimal moisture to the seeds thoughout the test
- Substrate: paper, sand or soil
- Temperature: maize 20-30°C (or alternating 30°C in light and 20°C in dark)
  wheat 20°C
- Light: cool white fluorescent - at least 8 h/day
- Duration: maize 7 days
  wheat 7 days

Tolerances

Tolerances are used to determine if the results for individual replicates within a test are consistent; and for comparing a test result with another test result. They are applicable to % germination with 400 seeds for:

- maximum tolerated ranges between replicates
- compatibility of tests

Seed vigour tests

Seed vigour tests are based on the simulation of environmental stress conditions encountered during storage or during field emergence.

Cold test-simulates early spring field conditions by providing high soil moistures and low soil temperature.

Accelerated aging test - seeds are stressed in temperatures of 40-45°C and near 100% relative humidity for varying lengths of time prior to a germination test.

Complex stress vigour test - seeds are soaked in sodium hypochlorite at a low temperature prior to a germination test. Seedlings are then measured and classified according to length.

Seedling vigour classification is similar to the standard germination test but normal seedlings are further classified as 'strong' and 'weak'.

Uses of seed germination/vigour tests

Routine seed germination/vigour investigations of seeds may be used for several purposes:

- Quality control programmes
- Indication of storage life
- Evaluation of effect of seed treatments and other critical operations
- Anticipation of potential problems
- Identification of good quality seed lots
- Consumer demand
- Labelling

Protocols for seed viability, germination and vigour tests

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Seed Viability Test

Tetrazolium seed viability test

Crop: Wheat
Sample: 400 seeds (4 replicates of 100 seeds)

213

Viable and non-viable wheat seeds stained with tetrazolium

214

Classification of viable and non-viable wheat seeds by distribution of tetrazolium staining

Procedure:

1) Tetrazolium salt is unstable in light, especially at higher temperatures so care should be taken in preparing the solution (1%). Heat water to no more than 60°C and place in an amber glass bottle or aluminium foil covered flask with the solid salt (4 g solid tetrazolium chloride to 400 ml distilled water). Agitate the solution until the solid dissolves. Allow to cool to ambient temperatures prior to use.

2) Soak seeds overnight in water (6-18 h).

3) Drain seeds and sub-section longitudinally through the embryo.

4) Immerse one half of each seed in a beaker containing the tetrazolium solution and incubate in the dark at 30°C for 2 to 6 hours; discard the remaining halves. Cover beakers with aluminium foil to keep out light.

5) Rinse seeds thoroughly after treatment and examine immediately, taking care not to expose seed to light before evaluation. Approximately 12 hours after incubation, the starch begins to hydrolyse and the residual chemical is reduced, causing deposition of the dye on all parts of the embryo, and masking the original staining.

Evaluation:

Classification is carried out according to the parts of the embryo stained based on the International Seed Testing Association (ISTA) rules. The maximum area of **un**stained tissue permitted includes the root area except for 2 root initials, and a third of the tips of the scutellum. The seed in Figure I is completely stained, and therefore, is viable. Figures II, III, and IV show the maximum area of unstained tissue allowed in viable seeds. Figure V illustrates a non-viable seed; with the unstained (necrotic) tissue at the centre of the scutellum indicating heat damage.

Result:

Seed viability test = % of viable seeds.

References

International Seed Testing Association. 1985. International Rules for Seed Testing 1985. *Seed Science and Technology* 13 (2): 299-520.

Seed Germination Test

Rolled paper towel seed germination test

Crop: Wheat
Sample: 400 seeds (4 replicates of 100 seeds)

215

Rolled paper towel germination test of wheat

Procedure:

1) Space 4 replicates of 100 seeds each uniformly on the upper halves of moist filter or blotter paper towels (50 x 25 cm).

2) Fold towels in the middle and cover seeds completely with the lower half folded upwards.

3) Roll towels and place 4 replicates in a plastic bag open at the top.

4) Place the plastic bag upright in an incubator at 20°C for 8 days.

Evaluation:

Determine numbers of normal seedlings, abnormal seedlings and ungerminated seeds for each replicate of 100 seeds.

Result:

The germination percentage is the mean number of normal seedlings in the four replicates, provided that the four replicates of a test are within the maximum tolerated range (see note on tolerances at the end of Annex). The mean percentage is rounded to the nearest whole number.

References

International Seed Testing Association 1985. International Rules for Seed Testing 1985. *Seed Science and Technology* 13 (2): 299-520.

Seed Germination Test

Sand/soil seed germination test

Crop: Wheat
Sample: 400 seeds (4 replicates of 100 seeds)

216

Soil germination test of wheat

Procedure:

1) Fill 4 trays with 4 cm of sand or soil.

2) Place 100 seeds at uniform intervals in each tray.

3) Place another 4 cm of sand or soil on top of the seeds.

4) Incubate seed trays at 20°C and evaluate after 8 days.

Evaluation:

Determine numbers of normal seedlings, abnormal seedlings and ungerminated seeds for each replicate of 100 seeds.

Result:

The germination percentage is the mean number of normal seedlings in the four replicates, provided that the four replicates of a test are within the maximum tolerated range (see note on tolerances at the end of Annex). The mean percentage is rounded to the nearest whole number.

References

International Seed Testing Association 1985. International Rules for Seed Testing 1985. *Seed Science and Technology* 13 (2): 299-520.

Note:

This test is recommended as a confirmation test for seed treated with fungicide and/or insecticide if paper towel tests indicate an unexpected high degree of toxicity. Some chemical products will be more toxic in rolled paper towel tests than in sand or soil.

Seed Vigour Test

Accelerated ageing test

Crop: Wheat
Sample: 400 seeds (4 replicates of 100 seeds)

217

Accelerated ageing vigour test of wheat

Procedure:

1) Place sufficient distilled water in plastic or glass containers to cover the bottom surface and maintain relative humidity near 100%. Place *one layer* of seeds in galvanised wire-mesh trays supported by a galvanized wire-mesh stand in each container. The number of seeds per container will depend on the size of the container.

2) Cover the containers with lids and completely immerse in a water bath at 45°C for 48 hours.

3) Remove samples from containers after the ageing period, and sow seeds on paper towels (50 x 25 cm).

4) Space 4 replicates of 100 seeds uniformly on the upper halves of moist filter or blotter paper towels.

5) Fold towels in the middle and cover seeds completely with the lower half folded upwards.

6) Roll towels and place the 4 replicates in a plastic bag open at the top.

7) Place the plastic bag upright in an incubator at 20°C for 8 days.

Evaluation:

Determine numbers of normal seedlings, abnormal seedlings, and ungerminated seeds for each replicate of 100 seeds.

Result:

Normal seedlings are considered to have been produced from seeds of acceptable vigour. The percentage of vigorous seeds is the mean number of normal seedlings in the four replicates. The mean percentage is rounded to the nearest whole number.

References

International Seed Testing Association. 1987. *Handbook of Vigor Testing Methods*. ISTA, Switzerland.

Seed Vigour Test

Complex stress vigour test

Crop: Wheat
Sample: 400 seeds (4 replicates of 100 seeds; with 5 sub-replicates of 20 seeds each)

Procedure:

1) Soak seeds in 0.009-0.15% sodium hypochlorite solution for 2 days @ 20°C.

2) Soak seeds in the same solution for a further 2 days @ 5°C.

3) Place seeds in a straight line on the upper halves of moist filter or blotter paper towels (50 x 25 cm), 25 seeds per towel. Place seeds with the radicle end pointed toward the bottom of the towel and the embryo side facing up.

4) Fold towels in the middle and cover seeds completely with the lower halves folded upwards.

5) Roll towels and place the 16 sub-replicates in 4 plastic bags open at the top.

6) Place bags upright in an incubator at 20°C for 8 days.

Evaluation:

1) Measure length of each of the ten longest seedlings for each replicate of 100 seeds and calculate the mean length.

2) Count the numbers of seeds, for each replicate of 100 seeds, in each of the following categories:
   a) length greater than 2/3 mean length of 10 longest,
   b) length between 1/3 and 2/3,
   c) length less than 1/3,
   d) ungerminated seeds.

Result:

Vigour score is presented as a value from 1-10 based on the number of seeds in the first category.

References

Barla-szabo, G. & Dolinka, B. (1988). Complex stressing vigor test: a new method for wheat and maize seeds. *Seed Science and Technology* 16: 63-73.

Note:

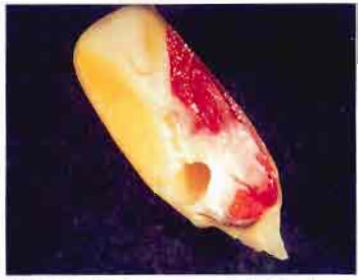
The procedure given here is the modified version of the published technique as followed by the National Institute of Agricultural Botany, Cambridge, UK.

Seed Viability Test

Tetrazolium seed viability test

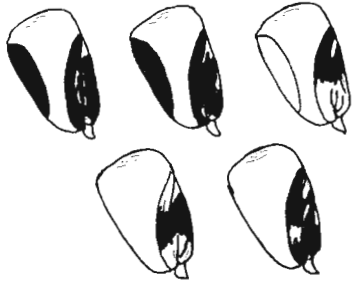
Crop: Maize
Sample: 400 seeds (4 replicates of 100 seeds)

218



Non-viable maize seed stained with tetrazolium

219



Classification of viable and non-viable maize seeds by distribution of tetrazolium staining

Procedure:

1) Tetrazolium salt is unstable in light, especially at higher temperatures so care should be taken in preparing the solution (1%). Heat water to no more than 60°C and place in an amber glass bottle or aluminium foil covered flask with the solid salt (4 g solid tetrazolium chloride to 400 ml distilled water). Agitate the solution until the solid dissolves. Allow to cool to ambient temperatures prior to use.

2) Soak seeds overnight in water (18 h).

3) Drain seeds and subsection longitudinally through the embryo.

4) Immerse one half of each seed in a beaker containing the tetrazolium solution and incubate in the dark at 30 C for 2 to 6 hours; discard the remaining halves. Cover beakers with aluminium foil to keep out light.

5) Rinse seeds thoroughly after treatment and examine immediately, taking care not to expose seed to light before evaluation. Approximately 12 hours after incubation, the starch begins to hydrolyse and the residual chemical is reduced, causing deposition of the dye on all parts of the embryo, and masking the original staining.

Evaluation:

Classification is carried out according to the parts of the embryo stained based on the International Seed Testing Association (ISTA) rules. The maximum area of **un**stained tissue permitted includes the primary root, and a third of the tips of the scutellum. The seed in Figure I is completely stained, and therefore, is viable. Figures II, III, and IV show the maximum area of unstained tissue allowed in viable seeds. Figure V illustrates a non-viable seed with the unstained tissue at the centre of the scutellum indicating heat damage.

Result:

Seed viability test = % of viable seeds.

References

International Seed Testing Association. 1985. International Rules for Seed Testing 1985. *Seed Science and Technology* 13 (2): 299-520.

Seed Germination Test

Rolled paper towel seed germination test

Crop: Maize

Sample: 400 seeds (4 replicates of 100 seeds with 2 sub-replicates of 50 seeds each)

220

Rolled paper towel germination test of maize

Procedure:

1) Space 8 sub-replicates of 50 seeds each uniformly on the upper halves of moist filter or blotter paper towels (50 x 25 cm). Place seeds with the radicle end pointed towards the bottom of the towel and the embryo end side up.

2) Fold towels in the middle and cover seeds completely with the lower halves folded upwards.

3) Roll towels and place the 8 sub-replicates in two plastic bags open at the top.

4) Place the plastic bags upright in an incubator at 25°C (or alternating 30°C in light and 20°C in dark) for 7 days.

Evaluation:

Determine numbers of normal seedlings, abnormal seedlings and ungerminated seeds for each replicate of 100 seeds.

Result:

The germination percentage is the mean number of normal seedlings in the four replicates, provided that the four replicates of a test are within the maximum tolerated range (see note on tolerances at the end of Annex). The mean percentage is rounded to the nearest whole number.

References

International Seed Testing Association 1985. International Rules for Seed Testing 1985. *Seed Science and Technology* 13 (2): 299-520.

Seed Germination Test

Sand/soil seed germination test

Crop: Maize
Sample: 400 seeds (4 replicates of 100 seeds with 2 sub-replicates of 50 seeds each)

221

Soil germination test with maize

Procedure:

1) Fill 8 trays with 4 cm of sand or soil.

2) Place 50 seeds at uniform intervals in each tray.

3) Place another 4 cm of sand or soil on top of the seeds.

4) Incubate seed trays at 25°C (or alternating 30°C in light and 20°C in dark) and evaluate after 7 days.

Evaluation:

Determine numbers of normal seedlings, abnormal seedlings and ungerminated seeds for each replicate of 100 seeds.

Result:

The germination percentage is the mean number of normal seedlings in the four replicates, provided that the four replicates of a test are within the maximum tolerated range (see note on tolerances at the end of Annex). The mean percentage is rounded to the nearest whole number.

References

International Seed Testing Association 1985. International Rules for Seed Testing 1985. *Seed Science and Technology* 13 (2): 299-520.

Note:

This test is recommended as a confirmation test for seed treated with fungicide and/or insecticide if paper towel tests indicate an unexpected high degree of toxicity. Some chemical products will be more toxic in rolled paper towel tests than in sand or soil.

Seed Vigour Test

Cold test - rolled paper towels - incubator

Crop: Maize

Sample: 400 seeds (4 replicates of 100 seeds; with 2 sub-replicates of 50 seeds each)

222

Rolled paper towel cold test of maize

Procedure:

1) Soak blotter paper towels (50 x 25 cm) in cold water or soak and chill to 10°C. Place 50 seeds uniformly on the upper halves of the moist filter or blotter paper towels with the radicle end pointed towards the bottom of the towel and the embryo side facing up.

2) Cover seeds with a thin layer of a sand-soil mixture (1 part sand to 1 part field soil). The soil should be screened through a 5 mm sieve before mixing with the sand.

3) Fold towels in the middle and cover the seeds completely with the lower half folded upwards.

4) Roll towels and place the 8 sub-replicates in two plastic bags open at the top.

5) Place the plastic bags upright in an incubator at 10°C.

6) After 7 days, transfer the bags to an incubator at 25°C (or alternating 30°C in light and 20°C in dark), and evaluate the test after 4 days.

Evaluation:

Determine numbers of normal seedlings, abnormal seedlings and ungerminated seeds for each replicate of 100 seeds.

Result:

Normal seedlings are considered to have been produced from seeds of acceptable vigour. The percentage of vigorous seeds is the mean number of normal seedlings in the four replicates. The mean percentage is rounded to the nearest whole number.

References

International Seed Testing Association. 1987. *Handbook of Vigor Testing Methods*. ISTA, Switzerland.

Seed Vigour Test

Cold test - soil - greenhouse

Crop: Maize
Sample: 400 seeds (4 replicates of 100 seeds with 2 sub-replicates of 50 seeds each)

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Soil cold test of maize on left, with normal soil germination test on right

Procedure:

1) Fill 4 trays with 4 cm of a sand-soil mixture (1 part sand to 1 part soil).

2) Place 50 seeds uniformly in each seed tray.

3) Cover seeds with another 4 cm of sand and soil mixture.

4) Water trays with cold water (10°C) until sand-soil mixture is at approximately 70% of its water holding capacity.

5) Place trays in an incubator at 10°C.

6) After 7 days, transfer the trays to an incubator at 25°C (or alternating 30°C in light and 20°C in dark).

7) Evaluate seedlings after a further 4 days.

Evaluation:

Determine numbers of normal seedlings, abnormal seedlings and ungerminated seeds for each replicate of 100 seeds.

Result:

Normal seedlings are considered to have been produced from seeds of acceptable vigour. The percentage of vigorous seeds is the mean number of normal seedlings in the four replicates. The mean percentage is rounded to the nearest whole number.

References

International Seed Testing Association. 1987. *Handbook of Vigor Testing Methods*. ISTA, Switzerland.

Seed Vigour Test

Accelerated ageing test

Crop: Maize
Sample: 400 seeds (4 replicates of 100 seeds)

224

Accelerated ageing vigour test of maize

Procedure:

1) Place sufficient distilled water in a plastic or glass recipient to cover the bottom surface and maintain relative humidity near 100%. Place one layer of seeds on top of a galvanised wire mesh tray supported by a galvanized wire-mesh stand in each container. The number of seeds per container will depend on the size of the container.

2) Cover the containers with lids and completely immerse in a water bath at 92°C for 96 hours.

3) Remove samples from containers after the ageing period, and sow seeds on paper towels (50 x 25 cm).

4) Space 8 sub-replicates of 50 seeds uniformly on the upper halves of moist filter or blotter towels. Place seeds with the radicle end towards the bottom of the towel and the embryo side facing upwards.

5) Fold towels in the middle and cover seeds completely with the lower half folded upwards.

6) Roll towels and place the 8 sub-replicates in two plastic bags open at the top.

7) Place the plastic bags upright in an incubator at 25°C (or alternating 30°C in light and 20°C in dark) for 7 days.

Evaluation:

Determine numbers of normal seedlings, abnormal seedlings, and ungerminated seeds for each replicate of 100 seeds.

Result:

Normal seedlings are considered to have been produced from seeds of acceptable vigour. The percentage of vigorous seeds is the mean number of normal seedlings in the four replicates. The mean percentage is rounded to the nearest whole number.

References

International Seed Testing Association. 1987. *Handbook of Vigor Testing Methods*. ISTA, Switzerland.

Seed Vigour Test

Complex stress vigour test

Crop: Maize
Sample: 400 seeds (4 replicates of 100 seeds; with 5 sub-replicates of 20 seeds each)

Procedure:

1) Soak seeds in 0.009-0.15% sodium hypochlorite solution for 2 days @ 25°C.

2) Soak seeds in the same solution for a further 2 days @ 5°C.

3) Place seeds in a straight line on the upper halves of moist filter or blotter paper towels (50 x 25 cm), 20 seeds per towel. Place seeds with the radicle end pointed toward the bottom of the towel and the embryo side facing up.

4) Fold towels in the middle and cover seeds completely with the lower halves folded upwards.

5) Roll towels and place the 20 sub-replicates in 5 plastic bags open at the top.

6) Place bags upright in an incubator at 25°C (or alternating 30°C in light and 20°C in dark) for 7 days.

Evaluation:

1) Measure length of each of the ten longest seedlings for each replicate of 100 seeds and calculate the mean length.

2) Count the numbers of seeds, for each replicate of 100 seeds, in each of the following categories:
   a) length greater than 2/3 mean length of 10 longest,
   b) length between 1/3 and 2/3,
   c) length less than 1/3,
   d) ungerminated seeds.

Result:

Vigour score is presented as a value from 1-10 based on number of seeds in the first category.

References

Barla-szabo, G. & Dolinka, B. (1988). Complex stressing vigor test: a new method for wheat and maize seeds. *Seed Science and Technology* 16: 63-73.

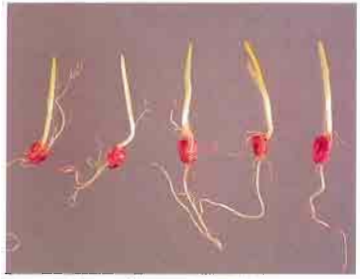
Note:

The procedure given here is the modified version of the published technique as followed by the CIMMYT Seed Health Unit.

225

Normal seedlings of wheat

226



Normal seedlings of maize

Normal seedlings

Seedlings showing potential for continued development into satisfactory plants when grown in good quality soil under optimum conditions. To be classified as normal, a seedling must conform with one of the following categories:

- **Intact seedlings** — seedlings with all their essential structures well developed, complete, in proportion and healthy.
- **Seedlings with slight defects** — seedlings showing certain slight defects of their essential structures, provided they show an otherwise satisfactory and balanced development comparable to that of intact seedlings of the same test.
- **Seedlings with secondary infection** — seedlings which would have conformed with 1 or 2 above, but which have been affected by fungi or bacteria from sources other than the parent seed.

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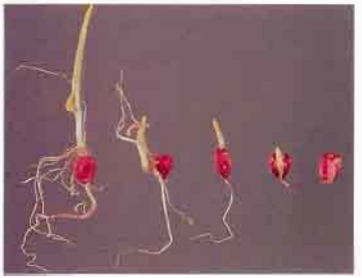
Abnormal seedlings of wheat

228



Abnormal seedlings of maize

Abnormal seedlings

Seedlings that do not show the potential to develop into a normal plant when grown in good quality soil and under favourable conditions of moisture, temperature and light. The following seedlings are classified as abnormal:

- **Damaged seedlings** — seedlings with any of the essential structures missing or so badly or irreparably damaged that balanced development cannot be expected.
- **Deformed or unbalanced seedlings** — seedlings with weak development or physiological disturbance or in which essential structures are deformed or out of proportion.
- **Decayed seedlings** — seedlings with any of their essential structures so diseased or decayed as a result of primary infection (i.e. from the parent seed) that normal development is prevented.

One or a combination of the following defects in maize or wheat renders it abnormal:

Radicle/seminal roots:
- stunted
- missing
- constricted
- with negative geotropism
- short and thick
- broken
- thin and weak
- glassy
- slow development
- split from the tip
- trapped in seed coat
- decayed as a result of primary infection

Seminal roots:
- only one or none

Note: seminal roots showing one or more of the above defects are abnormal; and at least two seminal roots must be present in *Triticum* spp.

Coleoptile:
- deformed
- with the tip damaged or missing
- tightly twisted
- split for more than 1/3 of the length from the tip
- damaged
- strongly bent over
- thin and weak
- decayed as a result of primary infection
- missing
- forming a loop or spiral
- split at the base

First leaf:
- extending less than half-way up the coleoptile
- missing
- shredded or otherwise deformed

Whole seedling:
- deformed
- coleoptile emerging before the radicle
- thin and weak
- decayed as a result of primary infection
- fractured
- yellow or white
- glassy

Seed Germination Evaluation

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Ungerminated seeds of wheat

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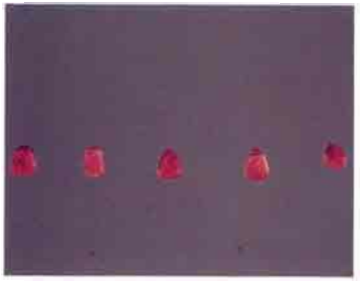

Ungerminated seeds of maize

Ungerminated seeds

Seeds which have not germinated by the end of the test period for a standard germination test.

Reference

International Seed Testing Association. 1985. International Rules for Seed Testing 1985. *Seed Science and Technology* 13 (2): 299-520.

Maximum tolerated ranges between replicates

This table indicates the maximum range (i.e. difference between highest and lowest) in germination percentage tolerable between replicates, allowing for random sampling variation of 2.5 %. To find the maximum tolerated range, calculate the average percentage, to the nearest whole number, of the four replicates. If necessary , form 100-seed replicates by combining the sub-replicates of 50 or 25 seeds which were closest together in the germinator. Locate the average in column 1 or 2 of the table and read the maximum tolerated range in column 3.

Average percentage germination		Maximum range (%)	Average percentage germination		Maximum range (%)
1	2	3	1	2	3
99	2	5	87 to 88	13 to 14	13
98	3	6	84 to 86	15 to 17	14
97	4	7	81 to 83	18 to 20	15
96	5	8	78 to 80	21 to 23	16
95	6	9	73 to 77	24 to 28	17
93 to 94	7 to 8	10	67 to 72	29 to 34	18
91 to 92	9 to 10	11	56 to 66	35 to 45	19
89 to 90	11 to 12	12	51 to 55	46 to 50	20

These tolerances are applicable only in the conditions defined. They are not, for example, suitable for comparing two test results from the same sample.

AGROVOC descriptors: *Zea mays*; maize; *Triticum*; wheats; seed testing; seedborne organisms; identification; laboratory diagnosis; disease control; quarantine

AGRIS category codes: F03

Dewey decimal classification: 631.521072

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