E

TC-EDC/Jan14/18
ORIGINAL: English
DATE: November 1, 2013

# INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

## ENLARGED EDITORIAL COMMITTEE

## Geneva, January 8 and 9, 2014

PARTIAL REVISION OF THE TEST GUIDELINES FOR CUCUMBER
(DOCUMENT TG/61/7)

*Document prepared by the Office of the Union*

*Disclaimer: this document does not represent UPOV policies or guidance*

1. At its forty-seventh session held in Nagasaki, Japan, from May 20 to 24, 2013, the Technical Working Party for Vegetables (TWV) considered the partial revision of the Test Guidelines for Cucumber on the basis of documents TG/61/7 (see document TWV/47/34 "Report", paragraph 72).

2. The structure of this document is as follows:



3. The proposed revisions are presented in the Annex to this document.

[Annex follows]

ANNEX

Proposal for a Revision of the Grouping Characteristics in Chapter 5.3

*Current wording:*

(a) Cotyledon: bitterness (characteristic 1)
(b) Plant: sex expression (characteristic 13)
(c) Ovary: color of vestiture (characteristic 15)
(c) Parthenocarpy (characteristic 16)
(d) Fruit: length (characteristic 17)
(e) Fruit: ground color of skin at market stage (characteristic 25)

*Proposed new wording:*

(a) Cotyledon: bitterness (characteristic 1)
(b) Plant: sex expression (characteristic 13)
(c) Ovary: color of vestiture (characteristic 15)
(c) Parthenocarpy (characteristic 16)
(d) Fruit: length (characteristic 17)
(e) Fruit: ground color of skin at market stage (characteristic 25)
(f) Resistance to *Cladosporium cucumerinum* (Ccu) (characteristic 44)
(g) Resistance to *Cucumber mosaic virus* (CMV) (characteristic 45)
(h) Resistance to Powdery mildew (*Podosphaera xanthii*) (Px) (characteristic 46)
(i) Resistance to Corynespora blight and target leaf spot (*Corynespora cassiicola*) (Cca) (characteristic 48)
(j) Resistance to *Cucumber vein yellowing virus* (CVYV) (characteristic 49)

Proposal for a Revision of the Chapter 7: Table of Characteristics
*Proposal to revise Characteristics 44 to 50*

*Current wording:*

| | | | | | | |
|---|---|---|---|---|---|---|
| **44.**<br>**(+)** | **Resistance to *Cladosporium cucumerinum* (Ccu)** | **Résistance à *Cladosporium cucumerinum* (Ccu)** | **Resistenz gegen *Cladosporium cucumerinum* (Ccu)** | **Resistencia a la *Cladosporium cucumerinum* (Ccu)** | | |
| **QL** | absent | absente | fehlend | ausente | Pepinex 69 | 1 |
| | present | présente | vorhanden | presente | Maketmore 76 | 9 |

*Proposed new wording:*

| | | | | | | |
|---|---|---|---|---|---|---|
| **44.**<br>**(+)** | **Resistance to *Cladosporium cucumerinum* (Ccu)** | **Résistance à *Cladosporium cucumerinum* (Ccu)** | **Resistenz gegen *Cladosporium cucumerinum* (Ccu)** | **Resistencia a la *Cladosporium cucumerinum* (Ccu)** | | |
| **QL** | absent | absente | fehlend | ausente | Cherubino, Frontera, Pepinex 69 | 1 |
| | present | présente | vorhanden | presente | Corona, Marketmore 76, Sheila | 9 |

*Current wording:*

| | | | | | | |
|---|---|---|---|---|---|---|
| **45.**<br>**(+)** | **Resistance to Cucumis Mosaic Virus (CMV)** | **Résistance au virus de la mosaïque du concombre** | **Resistenz gegen Gurkenmosaikvirus (CMV)** | **Resistencia al virus del mosaico del pepino (CMV)** | | |
| **QN** | susceptible | sensibilité | anfällig | susceptible | Gele Tros | 1 |
| | moderately resistant | résistance moyenne | mäßig resistent | intermedia | Gardon | 2 |
| | highly resistant | forte résistance | hochresistent | alta | Hokus, Naf | 3 |

*Proposed new wording:*

| | | | | | | |
|---|---|---|---|---|---|---|
| **45.**<br>**(+)** | **Resistance to *Cucumber mosaic virus* (CMV)** | **Résistance au virus de la mosaïque du concombre (CMV)** | **Resistenz gegen Gurkenmosaikvirus (CMV)** | **Resistencia al virus del mosaico del pepino (CMV)** | | |
| **QN** | susceptible | sensibilité | anfällig | susceptible | Bosporus, Corona, Ventura | 1 |
| | moderately resistant | résistance moyenne | mäßig resistent | intermedia | Capra, Gardon, Verdon | 2 |
| | highly resistant | forte résistance | hochresistent | alta | Naf, Picolino | 3 |

*Current wording:*

| | | | | | | |
|---|---|---|---|---|---|---|
| **46.**<br>**(+)** | **Resistance to powdery mildew (*Podosphaera xanthii*) (Sf)** | **Résistance à l'oïdium (*Podosphaera xanthii*) (Sf)** | **Resistenz gegen Echten Mehltau (*Podosphaera xanthii*) (Sf)** | **Resistencia al mildiú blanco (*Podosphaera xanthii*) (Sf)** | | |
| **QN** | susceptible | sensibilité | anfällig | susceptible | Corona | 1 |
| | moderately resistant | résistance moyenne | mäßig resistent | intermedia | Flamingo | 2 |
| | highly resistant | forte résistance | hochresistent | alta | Cordoba | 3 |

*Proposed new wording:*

| | | | | | | |
|---|---|---|---|---|---|---|
| **46.**<br>**(+)** | **Resistance to Powdery mildew (*Podosphaera xanthii*) (Px)** | **Résistance à l'oïdium (*Podosphaera xanthii*) (Px)** | **Resistenz gegen Echten Mehltau (*Podosphaera xanthii*) (Px)** | **Resistencia al mildiú blanco (*Podosphaera xanthii*) (Px)** | | |
| **QN** | susceptible | sensibilité | anfällig | susceptible | Corona, Ventura | 1 |
| | moderately resistant | résistance moyenne | mäßig resistent | intermedia | Flamingo | 2 |
| | highly resistant | forte résistance | hochresistent | alta | Aramon, Bella, Cordoba | 3 |

*Current wording:*

| | | | | | | |
|---|---|---|---|---|---|---|
| **47.**<br>**(+)** | **Resistance to downy mildew (*Pseudoperonospora cubensis*) (Pc)** | **Résistance au mildiou (*Pseudoperonospora cubensis*) (Pc)** | **Resistenz gegen Falschen Mehltau (*Pseudoperonospora cubensis*) (Pc)** | **Resistencia al mildiú velloso del pepino (*Pseudoperonospora cubensis* (Pc))** | | |
| **QN** | susceptible | sensibilité | anfällig | susceptible | Pepinex 69, SMR 58 | 1 |
| | moderately resistant | résistance moyenne | mäßig resistent | intermedia | Poinsett | 2 |
| | highly resistant | forte résistance | hochresistent | alta | | 3 |

*Proposed new wording:*

| | | | | | | |
|---|---|---|---|---|---|---|
| **47.**<br>**(+)** | **Resistance to Downy mildew (*Pseudoperonospora cubensis*) (Pcu)** | **Résistance au mildiou (*Pseudoperonospora cubensis*) (Pcu)** | **Resistenz gegen Falschen Mehltau (*Pseudoperonospora cubensis*) (Pcu)** | **Resistencia al mildiú velloso del pepino (*Pseudoperonospora cubensis* (Pcu)** | | |
| **QL** | absent | absente | fehlend | ausente | Pepinex 69, Wisconsin | 1 |
| | present | présente | vorhanden | presente | Poinsett 76 | 9 |

*Current wording:*

| | | | | | | |
|---|---|---|---|---|---|---|
| **48.**<br>**(+)** | **Resistance to Corynespora blight and target leaf spot (*Corynespora cassiicola*) (Cca)** | **Résistance à la pourriture corynespora et à la septoriose (*Corynespora cassiicola*) (Cca)** | **Resistenz gegen Corynespora-Blattfleckenkrank-heit (*Corynespora cassiicola*) (Cca)** | **Resistencia a la mancha foliar (*Corynespora cassiicola*) (Cca)** | | |
| **QL** | absent | absente | fehlend | ausente | Cerrucho, Goya, Pepinova | 1 |
| | present | présente | vorhanden | presente | Corona, Cumlaude, Edona | 9 |

*Proposed new wording:*

| | | | | | | |
|---|---|---|---|---|---|---|
| **48.**<br>**(+)** | **Resistance to Corynespora blight and target leaf spot (*Corynespora cassiicola*) (Cca)** | **Résistance à la pourriture corynespora et à la septoriose (*Corynespora cassiicola*) (Cca)** | **Resistenz gegen Corynespora-Blattfleckenkrankheit (*Corynespora cassiicola*) (Cca)** | **Resistencia a la mancha foliar (*Corynespora cassiicola*) (Cca)** | | |
| **QL** | absent | absente | fehlend | ausente | Bodega | 1 |
| | present | présente | vorhanden | presente | Corona, Cumlaude | 9 |

*Current wording:*

| | | | | | | |
|---|---|---|---|---|---|---|
| **49.**<br>**(+)** | **Resistance to Cucumber Vein Yellowing Virus (CVYV)** | **Résistance au virus du jaunissement des nervures du concombre** | **Resistenz gegen Cucumber Vein Yellowing Virus (CVYV)** | **Resistencia al virus de las venas amarillas del pepino (CVYV)** | | |
| **QL** | absent | absente | fehlend | ausente | Corona | 1 |
| | present | présente | vorhanden | presente | Tornac | 9 |

*Proposed new wording:*

| | | | | | | |
|---|---|---|---|---|---|---|
| **49.**<br>**(+)** | **Resistance to *Cucumber vein yellowing virus* (CVYV)** | **Résistance au virus du jaunissement des nervures du concombre (CVYV)** | **Resistenz gegen *Cucumber vein yellowing virus* (CVYV)** | **Resistencia al virus de las venas amarillas del pepino (CVYV)** | | |
| **QL** | absent | absente | fehlend | ausente | Corinda, Corona, Ventura | 1 |
| | present | présente | vorhanden | presente | Dina, Summerstar, Tornac | 9 |

*Current wording:*

| | | | | | | |
|---|---|---|---|---|---|---|
| **50.**<br>**(+)** | **Resistance to Zucchini Yellow Mosaic Virus (ZYMV)** | **Résistance au virus de la mosaïque jaune de la courgette** | **Resistenz gegen Zucchinigelb-mosaikvirus (ZYMV)** | **Resistencia al virus del mosaico amarillo del calabacín (ZYMV)** | | |
| **QL** | absent | absente | fehlend | ausente | Corona | 1 |
| | present | présente | vorhanden | presente | Dina | 9 |

*Proposed new wording:*

| | | | | | | |
|---|---|---|---|---|---|---|
| **50.**<br>**(+)** | **Resistance to *Zucchini yellow mosaic virus* (ZYMV)** | **Résistance au virus de la mosaïque jaune de la courgette (ZYMV)** | **Resistenz gegen Zucchinigelb-mosaikvirus (ZYMV)** | **Resistencia al virus del mosaico amarillo del calabacín (ZYMV)** | | |
| **QL** | absent | absente | fehlend | ausente | Corona, Hilton, Ventura | 1 |
| | present | présente | vorhanden | presente | Dina, Summerstar, Thunder | 9 |

Proposal for a Revision of the Chapter 8: Explanations on the Table of Characteristics
*Proposal to Include a Revised Format for Disease Resistance Characteristics under section 8.2*

(Current and Proposed New Wording are presented on opposite pages)

*Current wording:*

Ad. 44: Resistance to *Cladosporium cucumerinum* (Ccu)

Method

Maintenance of disease

| | |
|---|---|
| Type of medium: | PDA (Potato Dextrose Agar) |
| Special conditions: | 7-8 days in the dark at 20°C |
| Remarks: | The spore suspension should have a concentration of $0.5 \times 10^5$ spores/ml. To be kept for a maximum of 4 days in a refrigerator at 4°C. |

Preparation of inoculum: Scrape off the fungus from the PDA medium, collect in a beaker and filter through a cheese-cloth.

Raising the plants

| | |
|---|---|
| Sowing: | In potting soil or compost |
| Temperature: | 22/20°C (day/night) |
| Light: | At least 16 hours |
| Number of plants: | 30 plants per sample |

Inoculation

| | |
|---|---|
| Growth stage of plants: | The plants should have a first leaf with a diameter of 3 cm. |
| Method of inoculation: | Spray spore suspension on leaves |

Special conditions after inoculation

| | |
|---|---|
| Temperature: | 22/20°C (day/night) |
| Light: | At least 16 hours |
| Special conditions: | Plastic cover placed over the plants. The plastic cover is closed during the first three days and thereafter slightly opened during the daytime. |

Duration of test

| | |
|---|---|
| - From sowing to inoculation: | 12 days |
| - From inoculation to last reading: | 6-8 days |

Standard varieties: Resistance absent: Pepinex 69
Resistance present: Maketmore 76

*Proposed new wording:*

Ad. 44: Resistance to *Cladosporium cucumerinum* (Ccu)

| | |
|---|---|
| 1. Pathogen | *Cladosporium cucumerinum* |
| 2. Quarantine status | no |
| 3. Host species | *Cucumis sativus* (cucumber or gherkin) |
| 4. Source of inoculum | Naktuinbouw (NL) |
| 5. Isolate | natural; to be taken from any source of infection in the field |
| 6. Establishment isolate identity | expected reactions on resistant standard varieties |
| 7. Establishment pathogenicity | symptoms on susceptible standard varieties |
| 8. Multiplication inoculum | |
| 8.1 Multiplication medium | agar medium e.g.: Potato Dextrose Agar (PDA) |
| 8.2 Multiplication variety | - |
| 8.3 Plant stage at inoculation | - |
| 8.4 Inoculation medium | sterile demineralized water |
| 8.5 Inoculation method | scrape the Petri dishes and spread over new plates |
| 8.6 Harvest of inoculum | from 7-8 days old subcultures in the dark at 20°C |
| 8.7 Check of harvested inoculum | - |
| 8.8 Shelflife/viability inoculum | 4 days at 4°C |
| 9. Format of the test | |
| 9.1 Number of plants per genotype | at least 20 |
| 9.2 Number of replicates | 1 |
| 9.3 Control varieties | Cherubino, Frontera, Pepinex 69 (susceptible)<br>Corona, Marketmore 76, Sheila (resistant) |
| 9.4 Test design | e.g. after every 8 samples 16 resistant and 16 susceptible plants |
| 9.5 Test facility | - |
| 9.6 Temperature | 18 or 22/20°C day/night |
| 9.7 Light | at least 16 hours |
| 9.8 Season | - |
| 9.9 Special measures | make sure soil is not dry at time of inoculation; plastic tent closed day and night during first three days after inoculation; thereafter slightly opened during daytime |
| 10. Inoculation | |
| 10.1 Preparation inoculum | optional: add 0,01% Tween to spore suspension |
| 10.2 Quantification inoculum | $0.5*10^5$ -$0.5*10^6$ spores/mL |
| 10.3 Plant stage at inoculation | young cotyledon or first true leaf |
| 10.4 Inoculation method | spraying spore suspension |
| 10.5 First observation | 6 days post inoculation |
| 10.6 Second observation | 8 days post inoculation |
| 10.7 Final observations | 8 days post inoculation |
| 11. Observations | |
| 11.1 Method | visual, comparative |
| 11.2 Observation scale | |
| [1] absent: Frontera | brown lesions on cotyledons and plant death |
| [9] present: Corona | without symptoms, or with green lesions, or browning of the leaves |
| 11.3 Validation of test | on standards |
| 11.4 Off-types | maximum 1 out of 6-35 plants |
| 12. Interpretation of data in terms of UPOV characteristic states | QL |
| 13. Critical control points | temperature and humidity |

*Current wording:*

Ad. 45: Resistance to Cucumis Mosaic Virus (CMV)

Method

Maintenance of disease

| | |
|---|---|
| Type of medium: | On susceptible living plants |
| Remarks: | Greenhouse to be kept free from aphids |

Preparation of inoculum: Mix freshly infected leaves with water. Prepare a solution with a concentration of 1:15 (inoculum: water).

Raising the plants

| | |
|---|---|
| Sowing: | In potting soil or compost |
| Temperature: | 22/20°C (day/night) |
| Light: | At least 16 hours |
| Number of plants: | 30 plants per sample |

Inoculation

| | |
|---|---|
| Growth stage of plants: | Fully developed cotyledons |
| Method of inoculation: | Mechanical inoculation, by rubbing the cotyledons using carborundum powder. Carborundum powder to be washed away after inoculation. |

Special conditions after inoculation

| | |
|---|---|
| Temperature: | 22/20°C (day/night) |
| Light: | 16 hours |

Duration of test

| | |
|---|---|
| - From sowing to inoculation: | 6-7 days |
| - From inoculation to last reading: | 10-14 days |

Scheme of observation:

1. Susceptible

| | | |
|---|---|---|
| II | restricted growth, cotyledon slightly blistered, leaves completely mottled | Gele Tros |
| III | curled leaves, heavy mosaic symptoms over whole leaf | |

2. Moderately resistant

| | | |
|---|---|---|
| IV | curled leaves, slight mosaic symptoms | Gardon |
| V | slightly curled leaves, slight mosaic symptoms, many necrotic spots | |
| VI | leaves not curled, vague mosaic symptoms, few necrotic spots | |

3. Highly resistant

| | | |
|---|---|---|
| VII | very few virus symptoms, very few necrotic spots | |
| VIII | no symptoms | Hokus, Naf |

*Proposed new wording:*

Ad. 45: Resistance to *Cucumber mosaic virus* (CMV)

| | |
|---|---|
| 1. Pathogen | *Cucumber mosaic virus* |
| 2. Quarantine status | no |
| 3. Host species | *Cucumis sativus* (cucumber or gherkin) |
| 4. Source of inoculum | Naktuinbouw (NL), GEVES (FR) |
| 5. Isolate | e.g. UK 6 |
| 6. Establishment isolate identity | resistant and susceptible controls or ELISA dipstick (Agdia) |
| 7. Establishment pathogenicity | susceptible control inoculation |
| 8. Multiplication inoculum | |
| 8.1 Multiplication medium | on susceptible living plants |
| 8.2 Multiplication variety | susceptible control |
| 8.3 Plant stage at inoculation | cotyledons |
| 8.4 Inoculation medium | ice-cold Phosphate Buffer Solution +carborundum+ active charcoal |
| 8.5 Inoculation method | rubbing |
| 8.6 Harvest of inoculum | fresh symptomatic leaf |
| 8.7 Check of harvested inoculum | mock inoculation with PBS + carborundum |
| 8.8 Shelflife/viability inoculum | 8 hours at 4°C or on ice |
| 9. Format of the test | |
| 9.1 Number of plants per genotype | at least 30 |
| 9.2 Number of replicates | 3 |
| 9.3 Control varieties | Bosporus, Corona, Ventura (susceptible), Capra, Gardon, Verdon (moderately resistant), Naf, Picolino (highly resistant) |
| 9.4 Test design | e.g. replicates on different tablets in glasshouse |
| 9.5 Test facility | glasshouse or climatic chamber |
| 9.6 Temperature | 18-25°C /15-20°C day/night or 22°C constant |
| 9.7 Light | at least 16 hours |
| 9.8 Season | best results in Apr/May; Sep/Oct |
| 9.9 Special measures | keep glasshouse free of aphids |
| 10. Inoculation | |
| 10.1 Preparation inoculum | fresh leaf ground in cold PBS |
| 10.2 Quantification inoculum | - |
| 10.3 Plant stage at inoculation | Cotyledons, e.g.: 8 and 11 days after sowing |
| 10.4 Inoculation method | rubbing, rinse carborundum off |
| 10.5 First observation | 7 days post inoculation |
| 10.6 Second observation | 14 days post inoculation |
| 10.7 Final observations | 21 days post inoculation, first and second leaf symptoms; only needed when second observation is not decisive |
| 11. Observations | |
| 11.1 Method | visual estimate of mosaic severity on 1st leaf |
| 11.2 Observation scale | |
| [1] susceptible: 3, Corona, Ventura | mosaic; clear border between yellow and green |
| [1] susceptible: 4, Bosporus | heavy mottle; confluent chlorosis |
| [2] moderately resistant: 5, Gardon, Verdon | light mottle; chlorotic islands |
| [2] moderately resistant: 6, Capra | some chlorotic stippling |
| [3] highly resistant: 7, Naf, Picolino | no symptoms |
| 11.3 Validation of test | standards should conform to description; describe if different variation within standard should not exceed 1 scale point |
| 11.4 Off-types | 2 scale points difference with majority type, maximum 1 out of 6-35 plants |
| 12. Interpretation of data in terms of UPOV characteristic states | QN; [1] 3-4 susceptible, [2] 5-6 moderately resistant, [3] 7 highly resistant |

| | |
|---|---|
| 13. Critical control points | 1. Symptoms will develop from ring spot into mosaic (Ventura) or mottle (Gardon) or spots (Capra) Observation should focus on mature symptoms.<br>2. Aphids may transmit CMV as well as other viruses that may contaminate the CMV strain. Test should be in aphid-free compartment.<br>3. Growth inhibition is usually not strong enough to measure in young plants; severe growth inhibition is more likely caused by genetic aberration than by virus infection.<br>4. Leaf curling is not mentioned as a CMV symptom because leaf curling is usually caused by unbalanced growing conditions.<br>5. Replicates are intended to control the main source of variation. For CMV this is usually the amount of sunlight. Therefore, replicate tablets should represent the different levels of shading within one greenhouse compartment. |

*Current wording:*

Ad. 46: Resistance to powdery mildew *(Podosphaera xanthii)* (Sf)

Method

Maintenance of disease

| | |
|---|---|
| Type of medium: | On susceptible living plants |
| Preparation of inoculum: | Wash the spores from the infected leaves and prepare a suspension with a concentration of $10^5$ spores/ml. Filter the suspension through a cheese-cloth before infecting the plants. |

Raising the plants

| | |
|---|---|
| Sowing: | In potting soil or compost |
| Temperature: | 22/20°C (day/night) |
| Light: | At least 16 hours |
| Number of plants: | 30 plants per sample |

Inoculation

| | |
|---|---|
| Growth stage of plants: | Fully developed cotyledons |
| Method of inoculation: | Spray spore suspension on leaves on the first, second and fifth day after planting out. |

Special conditions after inoculation

| | |
|---|---|
| Temperature: | 20/20°C (day/night) |
| Light: | 16 hours |

Duration of test

| | |
|---|---|
| - From sowing to inoculation: | 7, 8 and 11 days |
| - From inoculation to last reading: | 12 days |

Scheme of observation

1. Susceptible: hypocotyls and cotyledons infected, first leaf strongly infected, high sporulation.
2. Moderately resistant: hypocotyls not infected, cotyledons and first leaf moderately infected with moderate sporulation, moderate colonization.
3. Highly resistant: hypocotyls and cotyledons not infected, first leaf very weakly or not infected, few colonies, very weak sporulation.

Standard varieties:
1. Susceptible: Corona
2. Moderately resistant: Flamingo
3. Highly resistant: Cordoba

*Proposed new wording:*

Ad. 46: Resistance to Powdery mildew *(Podosphaera xanthii)* (Px)

| | |
|---|---|
| 1. Pathogen | Powdery mildew *Podosphaera xanthii* (*Sphaerotheca fuliginea)* |
| 2. Quarantine status | no |
| 3. Host species | *Cucumis sativus* (cucumber or gherkin) |
| 4. Source of inoculum | natural or Naktuinbouw (NL) |
| 5. Isolate | natural; to be taken from any source of infection in the field |
| 6. Establishment isolate identity | expected reactions on resistant standard varieties |
| 7. Establishment pathogenicity | symptoms on susceptible standard varieties |
| 8. Multiplication inoculum | |
| 8.1 Multiplication medium | plants |
| 8.2 Multiplication variety | susceptible variety (e.g. Ventura) |
| 8.3 Plant stage at inoculation | first leaf appearing |
| 8.4 Inoculation medium | demineral water |
| 8.5 Inoculation method | spraying |
| 8.6 Harvest of inoculum | wash spores off from sporulating leaves with demineralized water,<br>option: add Tween20 at 5 µL (1 drop) /liter<br>filter with cheese-cloth. 0,75 ml/pl |
| 8.7 Check of harvested inoculum | count spores; target concentration is $1.10^5$ spores/ml |
| 8.8 Shelflife/viability inoculum | 15 minutes |
| 9. Format of the test | |
| 9.1 Number of plants per genotype | at least 20 |
| 9.2 Number of replicates | 1 |
| 9.3 Control varieties | Corona, Ventura (susceptible), Flamingo (moderately resistant), Aramon, Bella, Cordoba (highly resistant) |
| 9.4 Test design | - |
| 9.5 Test facility | - |
| 9.6 Temperature | 20°C constant |
| 9.7 Light | 16 hours |
| 9.8 Season | best results in autumn (Sep/Nov) |
| 9.9 Special measures | - |
| 10. Inoculation | |
| 10.1 Preparation inoculum | as above at 8.6 |
| 10.2 Quantification inoculum | $1.10^5$ spores/ml |
| 10.3 Plant stage at inoculation | cotyledon at 1st inoculation, first leaf at final inoculation |
| 10.4 Inoculation method | spraying, inoculation repeated on day 3, 5 and 6 after 1st |
| 10.5 First observation | 10 days post inoculation |
| 10.6 Second observation | - |
| 10.7 Final observations | 14 days post inoculation |
| 11. Observations | |
| 11.1 Method | visual, comparative; mainly on first leaf |
| 11.2 Observation scale | sporulation on cotyledons and hypocotyls; heavy sporulation on first leaf |
| [1] susceptible: Corona, Ventura | sporulation on cotyledons and hypocotyls; heavy sporulation on first leaf |
| [2] moderately resistant: Flamingo | no sporulation on hypocotyls,<br>moderate sporulation on cotyledons and the first leaf; |
| [3] highly resistant: Aramon, Bella, Cordoba | symptoms on cotyledons are disregarded<br>sometimes very light sporulation on first leaf |
| 11.3 Validation of test | on standard varieties |
| 11.4 Off-types | no more than 1 out of 6-35 plants |
| 12. Interpretation of data in terms of UPOV characteristic states | QN [1] susceptible, [2] moderately resistant, [3] highly resistant |
| 13. Critical control points | Some types of moderate resistance may break down at higher temperatures. |

*Current wording:*

Ad. 47: Resistance to downy mildew (*Pseudoperonospora cubensis*) (Pc)

Method

Maintenance of disease

| | |
|---|---|
| Type of medium: | On susceptible living plants |
| Preparation of inoculum: | Wash the spores from the infected leaves with cold distilled water and prepare a suspension. Suspension to be used immediately. |

Raising the plants

| | |
|---|---|
| Sowing: | In potting soil or compost |
| Temperature: | 22/20°C (day/night) |
| Light: | At least 16 hours |
| Number of plants: | 30 plants per sample |

Inoculation

| | |
|---|---|
| Growth stage of plants: | First two leaves fully developed |
| Method of inoculation: | Spray spore suspension on leaves. |

Special conditions after inoculation

| | |
|---|---|
| Temperature: | 22/20°C (day/night) |
| Light: | 16 hours |
| Relative humidity: | 100%, 48 hours after inoculation |
| Special conditions: | Plastic cover placed over the plants. The plastic cover is closed during the first three days and thereafter slightly opened during the daytime. |

Duration of test

| | |
|---|---|
| - From sowing to inoculation: | 20 days |
| - From inoculation to last reading: | ± 10 days |

Scheme of observations:

| | |
|---|---|
| Susceptible: | Large lesions with abundant spore production, leaf tissue becoming necrotic within 5 days. |
| Moderately resistant: | Medium lesions, period of tissue yellowing prolonged to beyond 10 days. |
| Highly resistant: | Small downy mildew lesions, round tissue in the center becoming necrotic, no visual spore production. |

| | | |
|---|---|---|
| Standard varieties: | Susceptible: | Pepinex 69, SMR 58 |
| | Moderately resistant: | Poinsett |
| | Highly resistant: | |

*Proposed new wording:*

Ad. 47: Resistance to Downy mildew (*Pseudoperonospora cubensis*) (Pcu)

| | |
|---|---|
| 1. Pathogen | Downy mildew (*Pseudoperonospora cubensis*) |
| 2. Quarantine status | no |
| 3. Host species | *Cucumis sativus* (cucumber or gherkin) |
| 4. Source of inoculum | natural |
| 5. Isolate | natural; to be taken from any source of infection in the field |
| 6. Establishment isolate identity | expected reactions on resistant standard varieties<br>Pepinex 69, Wisconsin (absent),<br>Poinsett 76 (present) |
| 7. Establishment pathogenicity | symptoms on susceptible standard varieties |
| 8. Multiplication inoculum | |
| 8.1 Multiplication medium | living plants |
| 8.2 Multiplication variety | susceptible variety |
| 8.3 Plant stage at inoculation | two leaves |
| 8.4 Inoculation medium | cold distilled water |
| 8.5 Inoculation method | spraying |
| 8.6 Harvest of inoculum | by washing a sporulating leaf |
| 8.7 Check of harvested inoculum | by counting the spores |
| 8.8 Shelflife/viability inoculum | - |
| 9. Format of the test | |
| 9.1 Number of plants per genotype | at least 20 |
| 9.2 Number of replicates | 1 |
| 9.3 Control varieties | Pepinex 69, Wisconsin (absent), Poinsett 76 (present) |
| 9.4 Test design | - |
| 9.5 Test facility | - |
| 9.6 Temperature | 22/20°C day/night |
| 9.7 Light | at least 16 hours |
| 9.8 Season | - |
| 9.9 Special measures | Keep 100% humidity for 24 hours. A plastic cover is placed over the plants. After 24 hours, the plastic cover is slightly opened during daytime. |
| 10. Inoculation | |
| 10.1 Preparation inoculum | by washing sporulating leaves |
| 10.2 Quantification inoculum | counting spores $10^3$ spores per ml |
| 10.3 Plant stage at inoculation | first two leaves fully developed |
| 10.4 Inoculation method | by spraying spore suspension on leaves |
| 10.5 First observation | 7 days post inoculation |
| 10.6 Second observation | - |
| 10.7 Final observations | 10 days post inoculation |
| 11. Observations | |
| 11.1 Method | visual, comparative |
| 11.2 Observation scale | |
| [1] absent: Pepinex 69, Wisconsin | large lesions with abundant sporulation, leaf tissue becoming necrotic within 5 days |
| [9] present: Poinsett76 | small circular lesions, necrotic in the center, sporulation visible macroscopically no highly resistant standard is available |
| 11.3 Validation of test | - |
| 11.4 Off-types | - |
| 12. Interpretation of data in terms of UPOV characteristic states | QL [1] absent, [9] present |
| 13. Critical control points | |

*Current wording:*

Ad. 48: Resistance to Corynespora blight and target leaf spot (*Corynespora cassiicola*) (Cca)

Method

Maintenance of disease

| | |
|---|---|
| Type of medium: | PDA (Potato Dextrose Agar) |
| Special conditions: | 12-14 days in the dark at 20°C |
| Remarks: | The spore suspension should have a concentration of 0.5 x $10^5$ spores/ml. To be kept for a maximum of 4 days in a refrigerator at 4°C |

Preparation of inoculum: Scrape off the fungus from the nutrient medium, collect in a beaker and filter through a cheese-cloth.

Raising the plants

| | |
|---|---|
| Sowing: | In potting soil or compost |
| Temperature: | 22/20°C (day/night) |
| Light: | At least 16 hours |
| Number of plants: | 30 plants per sample |

Inoculation

| | |
|---|---|
| Growth stage of plants: | The plants should have a first leaf with a diameter of 3 cm. |
| Method of inoculation: | Spray spore suspension on leaves |

Special conditions after inoculation

| | |
|---|---|
| Temperature: | 25/15°C (day/night) |
| Light: | At least 16 hours |
| Special conditions: | Plastic cover placed over the plants. The plastic cover is closed during the first three days and thereafter slightly opened during the daytime. |

Duration of test

| | |
|---|---|
| - From sowing to inoculation: | 12-13 days |
| - From inoculation to last reading: | 8-10 days |

Scheme of observation:

1. Susceptible

   a. cotyledons and first leaf dead, plant with greatly reduced growth
   b. cotyledons dead or strongly infected, first leaf weakly infected, plant with greatly reduced growth

2. Resistant

   a. cotyledons heavily infected, first leaf not infected, plant with normal growth
   b. cotyledons and first leaf not infected, plant with normal growth

Standard varieties:

Susceptible: Pepinova (1a) and Cerrucho, Goya (1b)
Resistant: Cumlaude, Edona (2a) and Corona (2b)

*Proposed new wording:*

Ad. 48: Resistance to Corynespora blight and target leaf spot (*Corynespora cassiicola*) (Cca)

| | |
|---|---|
| 1. Pathogen | *Corynespora cassiicola* (Target leaf spot) |
| 2. Quarantine status | no |
| 3. Host species | *Cucumis sativus* (cucumber or gherkin) |
| 4. Source of inoculum | Naktuinbouw (NL) |
| 5. Isolate | all sources of inoculums are equal |
| 6. Establishment isolate identity | expected reactions on resistant standard varieties |
| 7. Establishment pathogenicity | symptoms on susceptible standard varieties |
| 8. Multiplication inoculum | |
| 8.1 Multiplication medium | PDA at 20°C in darkness |
| 8.2 Multiplication variety | - |
| 8.3 Plant stage at inoculation | - |
| 8.4 Inoculation medium | demineralized water |
| 8.5 Inoculation method | scraping the Petri dishes and spread over new plates |
| 8.6 Harvest of inoculum | from 12-14 days old subcultures |
| 8.7 Check of harvested inoculum | - |
| 8.8 Shelflife/viability inoculum | max. 4 days at 4°C |
| 9. Format of the test | |
| 9.1 Number of plants per genotype | at least 20 |
| 9.2 Number of replicates | 1 |
| 9.3 Control varieties | Bodega, Pepinova (absent), Corona, Cumlaude (present) |
| 9.4 Test design | - |
| 9.5 Test facility | - |
| 9.6 Temperature | 25/15°C day/night or 23°C day/night in climatic chamber |
| 9.7 Light | at least 16 hours |
| 9.8 Season | best results obtained in February-April due to temperature |
| 9.9 Special measures | make sure soil is not dry at time of inoculation; plastic tent closed day and night 3 days post inoculation, closed only in night >3 days post inoculation |
| 10. Inoculation | |
| 10.1 Preparation inoculum | filter through cheesecloth; add 0,01% Tween to spore suspension |
| 10.2 Quantification inoculum | $0{,}5x10^5$ spores/ml |
| 10.3 Plant stage at inoculation | diameter first true leaf around 3 cm<br>transplant on day 7, then inoculate on day 12 |
| 10.4 Inoculation method | spraying spore suspension |
| 10.5 First observation | 8 days post inoculation |
| 10.6 Second observation | - |
| 10.7 Final observations | 8-11 days post inoculation |
| 11. Observations | |
| 11.1 Method | visual; comparative; mainly on cotyledon and first leaf |
| 11.2 Observation scale | |
| [1] highly susceptible: 1, Bodega | cotyledons dead, first leaves dead, growth retardation |
| [1] susceptible: 2, Pepinova | cotyledons dead or covered with lesions, first leaves with lesions, growth retardation |
| [9] resistant: 3, Cumlaude | cotyledons with a few lesions, first leaf with no or sometimes a few lesions |
| [9] highly resistant: 4, Corona | cotyledons without lesions; first leaf without lesions |
| 11.3 Validation of test | standards should conform to description; describe if different |
| 11.4 Off-types | maximum 1 out of 6-35 plants |
| 12. Interpretation of data in terms of UPOV characteristic states | QL [1] 1-2 absent, [9] 3-4 present |
| 13. Critical control points | - |

*Current wording:*

Ad. 49: Resistance to Cucumber Vein Yellowing Virus (CVYV)

Method

Maintenance of isolate

| | |
|---|---|
| Type of medium: | On susceptible living plants |
| Special conditions: | Fresh inoculum, or inoculum which has been stored for a maximum of 3 months at -20˚C |

Execution of test

| | |
|---|---|
| Growth stage of plants: | Appearance of first leaf |
| Temperature: | 16 to 30˚C |
| Light: | 16 hours |
| Growing method: | Greenhouse |
| Method of inoculation: | Mechanical, by rubbing of cotyledons |
| Duration of test: | From inoculation to reading: 14 days |
| Number of plants tested: | At least 15 plants |
| Standard varieties: | Susceptible: Corona<br>Resistant: Tornac |
| Remark: | Resistant varieties may have a slight discoloration of the veins of older leaves |

*Proposed new wording:*

Ad. 49: Resistance to *Cucumber vein yellowing virus* (CVYV)

| | |
|---|---|
| 1. Pathogen | *Cucumber vein yellowing virus* |
| 2. Quarantine status | no |
| 3. Host species | *Cucumis sativus* (cucumber or gherkin) |
| 4. Source of inoculum | Naktuinbouw (NL) |
| 5. Isolate | e.g. KB18 |
| 6. Establishment isolate identity | resistant and susceptible controls |
| 7. Establishment pathogenicity | susceptible control inoculation |
| 8. Multiplication inoculum | |
| 8.1 Multiplication medium | leaf |
| 8.2 Multiplication variety | susceptible variety (e.g. Corinda) |
| 8.3 Plant stage at inoculation | cotyledons / appearance of first leaf |
| 8.4 Inoculation medium | leaf in ice-cold PBS + carborundum |
| 8.5 Inoculation method | rubbing |
| 8.6 Harvest of inoculum | freeze-dried leaf |
| 8.7 Check of harvested inoculum | - |
| 8.8 Shelflife/viability inoculum | 8 hours at 4°C or on ice |
| 9. Format of the test | |
| 9.1 Number of plants per genotype | at least 30 |
| 9.2 Number of replicates | 1 |
| 9.3 Control varieties | Corinda, Corona, Ventura (susceptible), Dina, Summerstar, Tornac (resistant) |
| 9.4 Test design | - |
| 9.5 Test facility | greenhouse |
| 9.6 Temperature | 16-30°C |
| 9.7 Light | at least 16 hours |
| 9.8 Season | best results in Apr/May; Sep/Oct |
| 9.9 Special measures | 12.000 lux suggested; keep glasshouse free of aphids |
| 10. Inoculation | |
| 10.1 Preparation inoculum | fresh leaf ground in 0.03 M phosphate buffer + carborundum + active coal |
| 10.2 Quantification inoculum | - |
| 10.3 Plant stage at inoculation | cotyledons |
| 10.4 Inoculation method | rubbing, option: rinse carborundum off to prevent leaf damage |
| 10.5 First observation | 7 days post inoculation; cotyledon symptoms |
| 10.6 Second observation | 14 days post inoculation; first leaf symptoms |
| 10.7 Final observations | 21 days post inoculation, first and second leaf symptoms |
| 11. Observations | |
| 11.1 Method | visual; comparative; mainly on first leaf |
| 11.2 Observation scale | |
| [1] susceptible: 3, Corinda, Corona | mosaic; clear border between yellow and green |
| [1] susceptible: 4, Ventura | heavy mottle; confluent chlorosis |
| [9] resistant: 5, Dina | light mottle; chlorotic islands |
| [9] resistant: 6, Summerstar | some chlorotic stippling |
| [9] resistant: 7, Tornac | no symptoms |
| 11.3 Validation of test | Standards should conform to description; describe if different. Variation within standard should not exceed 1 scale point |
| 11.4 Off-types | maximum 1 out of 6-35 plants |
| 12. Interpretation of data in terms of UPOV characteristic states | QL; [1] 3-4 absent, [9] 5-7 present |
| 13. Critical control points | Resistant varieties may have a slight discoloration of the veins of older leaves. |

*Current wording:*

Ad. 50: Resistance to Zucchini Yellow Mosaic Virus (ZYMV)

Method

Maintenance of isolate

| | |
|---|---|
| Type of medium: | On susceptible living plants |
| Special conditions: | Fresh inoculum, or inoculum which has been stored for a maximum of 6 months at - 20˚C |

Execution of test

| | |
|---|---|
| Growth stage of plants: | Appearance of first leaf |
| Temperature: | 23 to 25˚C day and night |
| Light: | 16 hours |
| Growing method: | Greenhouse |
| Method of inoculation: | Mechanical, by rubbing of cotyledons |
| Duration of test: | From inoculation to reading: 14 days |
| Number of plants tested: | At least 15 plants |
| Standard varieties: | Susceptible: Corona<br>Resistant: Dina |
| Remark: | Resistant varieties may have a slight discoloration of the veins of older leaves.<br>Susceptible varieties have systemic mosaic symptoms. |

*Proposed new wording:*

Ad. 50: Resistance to *Zucchini yellow mosaic virus* (ZYMV)

| | |
|---|---|
| 1. Pathogen | *Zucchini yellow mosaic virus* |
| 2. Quarantine status | no |
| 3. Host species | *Cucumis sativus* (cucumber or gherkin) |
| 4. Source of inoculum | Naktuinbouw (NL) |
| 5. Isolate | e.g. CU61 |
| 6. Establishment isolate identity | resistant and susceptible controls; |
| 7. Establishment pathogenicity | susceptible control inoculation |
| 8. Multiplication inoculum | |
| 8.1 Multiplication medium | leaf |
| 8.2 Multiplication variety | susceptible control |
| 8.3 Plant stage at inoculation | cotyledons / appearance of first leaf |
| 8.4 Inoculation medium | ice-cold PBS + carborundum |
| 8.5 Inoculation method | rubbing |
| 8.6 Harvest of inoculum | fresh or dried leaf |
| 8.7 Check of harvested inoculum | |
| 8.8 Shelflife/viability inoculum | 8 hours at 4°C or on ice |
| 9. Format of the test | |
| 9.1 Number of plants per genotype | at least 30 |
| 9.2 Number of replicates | 1 |
| 9.3 Control varieties | Corona, Hilton, Ventura (susceptible), Dina, Summerstar, Thunder (resistant) |
| 9.4 Test design | - |
| 9.5 Test facility | greenhouse or climatic chamber |
| 9.6 Temperature | 18-25°C /15-25°C day/night |
| 9.7 Light | at least 16 hours |
| 9.8 Season | best results in Apr/May; Sep/Oct |
| 9.9 Special measures | 12.000 lux suggested; keep glasshouse free of aphids |
| 10. Inoculation | |
| 10.1 Preparation inoculum | fresh leaf ground in cold PBS |
| 10.2 Quantification inoculum | - |
| 10.3 Plant stage at inoculation | cotyledons / appearance of first leaf -(e.g. 8 days; repeat 3 days later) |
| 10.4 Inoculation method | rubbing, rinse carborundum off |
| 10.5 First observation | 7 - 14 days post inoculation; cotyledon symptoms |
| 10.6 Second observation | 14 - 21 days post inoculation; first leaf symptoms |
| 10.7 Final observations | 21 days post inoculation, first and second leaf symptoms |
| 11. Observations | |
| 11.1 Method | visual; comparative, mainly on first leaf |
| 11.2 Observation scale | |
| [1] absent: 4, Corona, Ventura | mosaic; leaf deformation |
| [1] absent: 5, Hilton | mosaic; weak leaf deformation |
| [9] present: 6, Thunder | weak mottle |
| [9] present: 7, Dina, Summerstar | vein necrosis |
| 11.3 Validation of test | Standards should conform to description; describe if different. Variation within standard should not exceed 1 scale point |
| 11.4 Off-types | 2 scale points difference with most present type, maximum 1 out of 30 plants |
| 12. Interpretation of data in terms of UPOV characteristic states | QL: [1] 4-5 absent, [9] 6-7 present |
| 13. Critical control points | Resistant varieties may have a slight discoloration of the veins of older leaves. Susceptible varieties have systemic mosaic symptoms. |

Proposal for a Revision of the Chapter 10 “Technical Questionnaire”
*Section 5: TQ characteristics selected from the Table of Characteristics*

To add an option “Not tested” to characteristics 44, 45, 46, 48, 49 to Section 5:

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

| | Characteristics | Example Varieties | Note |
|---|---|---|---|
| | […] | | |
| **5.9 (44)** | **Resistance to *Cladosporium cucumerinum* (Ccu)** | | |
| | absent | Cherubino, Frontera, Pepinex 69 | 1[ ] |
| | present | Corona, Marketmore 76, Sheila | 9[ ] |
| | not tested | | [ ] |
| **5.10 (45)** | **Resistance to *Cucumber mosaic virus* (CMV)** | | |
| | susceptible | Bosporus, Corona, Ventura | 1[ ] |
| | moderately resistant | Capra, Gardon, Verdon | 2[ ] |
| | highly resistant | Naf, Picolino | 3[ ] |
| | not tested | | [ ] |
| **5.11 (46)** | **Resistance to Powdery mildew (*Podosphaera xanthii*) (Px)** | | |
| | susceptible | Corona, Ventura | 1[ ] |
| | moderately resistant | Flamingo | 2[ ] |
| | highly resistant | Aramon, Bella, Cordoba | 3[ ] |
| | not tested | | [ ] |
| **5.12 (48)** | **Resistance to Corynespora blight and target leaf pot (*Corynespora cassiicola*) (Cca)** | | |
| | absent | Bodega | 1 [ ] |
| | present | Corona, Cumlaude | 9 [ ] |
| | not tested | | [ ] |
| **5.13 (49)** | **Resistance to *Cucumber vein yellowing virus* (CVYV)** | | |
| | absent | Corinda, Corona, Ventura | 1 [ ] |
| | present | Dina, Summerstar, Tornac | 9 [ ] |
| | not tested | | [ ] |

*Section 7: Addition of new characteristics under 7.3.1*

To add the following to Section 7 “Additional information which may help in the examination of the variety”:

7.3.1 Resistance to pests and diseases (please specify races/strains if possible)

| | | absent | present | not tested |
|---|---|---|---|---|
| (a) | Resistance to Downy mildew (*Pseudoperonospora cubensis*) (Pcu) (char. 47) | [ ] | [ ] | [ ] |
| (b) | Resistance *to Zucchini yellow mosaic virus* (ZYMV) (char. 50) | [ ] | [ ] | [ ] |

[End of Annex and of document]