TWV/47/29
ORIGINAL: English
DATE: April 22, 2013

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

Geneva

TECHNICAL WORKING PARTY FOR VEGETABLES

Forty-Seventh Session
Nagasaki, Japan, May 20 to 24, 2013

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

Document prepared by experts from the Netherlands

1. The purpose of this document is to present the proposals for the partial revision of the Test Guidelines for Cucumber (document TG/61/7).

2. The following changes are proposed:

(a) Revision of grouping characteristics, including the behavior against pathogens

(b) a revised format for disease resistance characteristics according to the explanations for disease resistance characteristics in Test Guidelines and eventual new proposal to update the protocol

- Chapter 5: Grouping of Varieties and Organization of the Growing Trial
- Chapter 7: Table of Characteristics
- Chapter 8: Explanations on the Table of Characteristics
  - 8.2 Explanations for individual characteristics
- Chapter 10 : Technical Questionnaire
  - Paragraph 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).

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 *Cladosporiumcucumerinum* (Ccu) (characteristic 44)
(g) Resistance to *Cucumber mosaic virus* (CMV) (characteristic 45)
(h) Resistance to powdery mildew (*Podosphaeraxanthii*) (Sf) (characteristic 46)
(i) Resistance to Corynespora blight and target leaf spot (*Corynesporacassiicola*) (Cca) (characteristic 48)
(j) Resistance to *Cucumber vein yellowing virus* (CVYV) (characteristic 49)

Proposal to revise Characteristics 44 to 47

Current wording:

44. **(+)**	**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. **(*)** **(+)**	**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	Marketmore 76	9

Current wording:

45. **(+)**	**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. **(*)** **(+)**	**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	Ventura	1
	moderately resistant	résistance moyenne	mäßig resistent	intermedia	Gardon, Verdon	2
	highly resistant	forte résistance	hochresistent	alta	Naf, Picolino	3

Current wording:

46. **(+)**	**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. **(*)** **(+)**	**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	1
	moderately resistant	résistance moyenne	mäßig resistent	intermedia	Flamingo	2
	highly resistant	forte résistance	hochresistent	alta	Cordoba	3

Current wording:

47. **(+)**	**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. **(+)**	**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 76	2
	highly resistant	forte résistance	hochresistent	alta		3

Current wording:

48. **(+)**	**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. **(*)** **(+)**	**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. **(+)**	**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. **(*)** **(+)**	**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	Corona	1
	present	présente	vorhanden	presente	Tornac	9

Current wording:

50. **(+)**	**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. **(+)**	**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	1
	present	présente	vorhanden	presente	Dina	9

Proposal to Include a Revised Format for Disease Resistance Characteristics
(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 0.5×10^5 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	*Cucumissativus* (cucumber or gherkin)
4. Source of inoculum	Naktuinbouw (NL)
5. Isolate	
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	
9.3 Control varieties	Frontera, Cherubino, Pepinex 69 (Susceptible) Corona, Sheila, Marketmore 76 (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 d/n
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 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 dpi
10.6 Second observation	8 dpi
10.7 Final observations	8 dpi
11. Observations	
11.1 Method	Visual, comparative
11.2 Observation scale	
[1] Susceptible: Frontera	brown lesions on cotyledons and plant death
[9] Resistant: 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 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	*Cucumissativus* (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 h 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	Corona, Ventura, Bosporus (Susceptible), Gardon, Verdon, Capra (Intermediate Resistant), Picolino, Naf
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 d/n or 22°C constant
9.7 Light	at least 16h
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 d after sowing
10.4 Inoculation method	rubbing, rinse carborundum off
10.5 First observation	7 dpi
10.6 Second observation	14 dpi
10.7 Final observations	21 dpi, 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, Ventura, Corona	mosaic; clear border between yellow and green
[1] Susceptible: 4, Bosporus	heavy mottle; confluent chlorosis
[2] Moderately resistant: 5, Verdon, Gardon	light mottle; chlorotic islands
[2] Moderately resistant: 6, Capra	some chlorotic stippling
[3] Highly resistant: 7, Picolino, Naf	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
12. Interpretation of data in terms of UPOV characteristic states	QN; [1] 3-4; [2] 5-6; [3] 7
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. 2. Aphids may transmit CMV as well as other viruses that may contaminate the CMV strain. Test should be in aphid-free compartment 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 4. Leaf curling is not mentioned as a CMV symptom because leaf curling is usually caused by unbalanced growing conditions. 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	
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, Option: add Tween20 at 5 µL (1 drop) /liter 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	
9.3 Control varieties	Ventura, Corona (Susceptible) Flamingo (IR); Bella, Aramon, Cordoba (Resistant)
9.4 Test design	
9.5 Test facility	
9.6 Temperature	20°C constant
9.7 Light	16 h
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 dpi
10.6 Second observation	14 dpi
10.7 Final observations	
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: Ventura, Corona	Sporulation on cotyledons and hypocotyls; heavy sporulation on first leaf
[2] Moderately resistant: Flamingo	No sporulation on hypocotyls, Moderate sporulation on cotyledons and the first leaf;
[3] Highly resistant: Bella, Aramon, ... Cordoba:	Symptoms on cotyledons are disregarded. Sometimes very light sporulation on first leaf.
11.3 Validation of test	on standard varieties
11.4 Off-types	No more than 1 of 6-35 plants
12. Interpretation of data in terms of ..... UPOV characteristic states	QN [1] susceptible; [2] intermediate; [3] resistant
13. Critical control points	Some types of intermediate 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*) (Pc)

1. Pathogen	Downy mildew (*Pseudoperonospora cubensis*)
2. Quarantine status	No
3. Host species	*Cucumissativus* (cucumber or gherkin)
4. Source of inoculum	
5. Isolate	
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	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	
9.3 Control varieties.....	Pepinex 69, SMR 58 (susceptible), Poinsett 76 (intermediate resistant)
9.4 Test design	
9.5 Test facility	
9.6 Temperature	22/20°C d/n
9.7 Light	at least 16h
9.8 Season.....	
9.9 Special measures	keep 100% humidity for 24 h. A plastic cover is placed over the plants. After 24 h, 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 dpi
10.6 Second observation.....	10 dpi
10.7 Final observations	
11. Observations	
11.1 Method.....	Visual, comparative
11.2 Observation scale	
[1] Susceptible: Pepinex 69, SMR58	large lesions with abundant sporulation, leaf tissue becoming necrotic within 5 days
[9] Moderately resistant: Poinsett76	small circular lesions, necrotic in the center, sporulation visible macroscopically no highly resistant standard is available
[9] Highly resistant:	On standards
11.3 Validation of test	
11.4 Off-types	
12. Interpretation of data in terms of UPOV characteristic states	QL [1] susceptible; [9] moderately and highly resistant
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	
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 d old subcultures
8.7 Check of harvested inoculum	
8.8 Shelflife/viability inoculum	max. 4 d at 4°C
9. Format of the test	
9.1 Number of plants per genotype	at least 20
9.2 Number of replicates	
9.3 Control varieties	Pepinova, Cerrucho, Goya (Susceptible); Corona, Cumlaude, Edona (Resistant)
9.4 Test design	
9.5 Test facility	
9.6 Temperature	25/15°C d/n or 23°Cd/n in climatic chamber
9.7 Light	at least 16h
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 dpi, closed only in night >3 dpi
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
	transplant on day 7, then inoculate on day 12
10.4 Inoculation method	spraying spore suspension
10.5 First observation	8-11 days
10.6 Second observation	
10.7 Final observations	
11. Observations	
11.1 Method	Visual; comparative; mainly on cotyledon and first leaf
11.2 Observation scale	
[1] 1, Highly susceptible: Bodega	Cotyledons dead, first leaves dead, growth retardation
[1] 2, Susceptible: Cerrucho	Cotyledons dead or covered with lesions, first leaves with lesions, growth retardation
[9] 3, Resistant: Cumlaude	Cotyledons with a few lesions, first leaf with no or sometimes a few lesions
[9] 4, Highly resistant: 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 of 6-35 plants
12. Interpretation of data in terms of UPOV characteristic states	QL
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 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
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. Korinda)
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 h 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	
9.3 Control varieties	Corona, Korinda, Ventura (Susceptible), Summerstar, Dina, Tornac (Resistant)
9.4 Test design	
9.5 Test facility	greenhouse
9.6 Temperature	16-30°C
9.7 Light	16h at least
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 charcoal
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 dpi; cotyledon symptoms
10.6 Second observation	14 dpi; first leaf symptoms
10.7 Final observations	21 dpi, first and second leaf symptoms
11. Observations	
11.1 Method	Visual; comparative; mainly on first leaf
11.2 Observation scale	
[1] Susceptible 3. Korinda, 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	
12. Interpretation of data in terms of UPOV characteristic states	QL; [1] 3-4, Susceptible; [9] 5-7, Resistant
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 Resistant: Dina
Remark:	Resistant varieties may have a slight discoloration of the veins of older leaves. 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
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 h 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	
9.3 Control varieties	Corona, Ventura, Hilton (Susceptible), Thunder, Summerstar, Dina (Resistant)
9.4 Test design	
9.5 Test facility	greenhouse or climatic chamber
9.6 Temperature	18-25°C /15-25°C d/n
9.7 Light	at least 16h
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. 8d.; repeat 3 d later)
10.4 Inoculation method	rubbing, rinsecarborundum off
10.5 First observation	7 - 14 dpi; cotyledon symptoms
10.6 Second observation	14 - 21 dpi; first leaf symptoms
10.7 Final observations	21 dpi, first and second leaf symptoms
11. Observations	
11.1 Method	Visual. Comparative, mainly on first leaf
11.2 Observation scale	
[1] Susceptible: 4. Ventura, Corona	Mosaic; leaf deformation
[1] Susceptible: 5. Hilton	Mosaic; weak leaf deformation
[9] Resistant: 6. Thunder	Weak mottle
[9] Resistant: 7. Summerstar, Dina	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 majority type
12. Interpretation of data in terms of UPOV characteristic states	QL: [1]4-5: Susceptible; [9] 6-7: Resistant
13. Critical control points	Resistant varieties may have a slight discoloration of the veins of older leaves. Susceptible varieties have systemic mosaic symptoms.

Proposed changes to Chapter 10 “Technical Questionnaire”

To add the following characteristics to Chapter TQ 5:

Resistance to *Cladosporiumcucumerinum* (Ccu) (characteristic 44)
Resistance to *Cucumber mosaic virus* (CMV) (characteristic 45)
Resistance to powdery mildew (*Podosphaeraxanthii*) (Sf) (characteristic 46)
Resistance to Corynespora blight and target leaf spot (*Corynesporacassiicola*) (Cca) (characteristic 48)
Resistance to *Cucumber vein yellowing virus* (CVYV) (characteristic 49)

[End of Annex and of document]