

TWV/47/29

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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]

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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					

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:

highly resistant

forte résistance

45.	Resistance to Cucumis Mosaic Virus (CMV)	Résistance au virus de la mosaïque du	Resistenz gegen Gurkenmosaikvirus	Resistencia al virus del mosaico del pepino		
(+)		concombre	(CMV)	(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:					
	Current wording: Resistance to powdery	Résistance à l'oïdium	Resistenz gegen	Resistencia al mildiú		
46.		Résistance à l'oïdium (Podosphaera xanthii) (Sf)	Resistenz gegen Echten Mehltau (<i>Podosphaera xanthii</i>) (Sf)	Resistencia al mildiú blanco (<i>Podosphaera</i> <i>xanthii</i>) (Sf)		
46. (+)	Resistance to powdery mildew (<i>Podosphaera</i>	(Podosphaera xanthii)	Echten Mehltau (Podosphaera xanthii)	blanco (Podosphaera	Corona	1
46. (+)	Resistance to powdery mildew (<i>Podosphaera</i> xanthii) (Sf)	(Podosphaera xanthii) (Sf)	Echten Mehltau (Podosphaera xanthii) (Sf)	blanco (<i>Podosphaera</i> xanthii) (Sf)	Corona Flamingo	1 2
46. (+)	Resistance to powdery mildew (Podosphaera xanthii) (Sf)	(<i>Podosphaera xanthii</i>) (Sf) sensibilité	Echten Mehltau (Podosphaera xanthii) (Sf) anfällig	blanco (Podosphaera xanthii) (Sf) susceptible		
16. (+) QN	Resistance to powdery mildew (Podosphaera xanthii) (Sf) susceptible moderately resistant	(Podosphaera xanthii) (Sf) sensibilité résistance moyenne	Echten Mehltau (Podosphaera xanthii) (Sf) anfällig mäßig resistent	blanco (Podosphaera xanthii) (Sf) susceptible intermedia	Flamingo	2
46. (+) QN	Resistance to powdery mildew (Podosphaera xanthii) (Sf) susceptible moderately resistant highly resistant	(Podosphaera xanthii) (Sf) sensibilité résistance moyenne forte résistance	Echten Mehltau (Podosphaera xanthii) (Sf) anfällig mäßig resistent	blanco (Podosphaera xanthii) (Sf) susceptible intermedia	Flamingo	2
46. (+) QN	Resistance to powdery mildew (Podosphaera xanthii) (Sf) susceptible moderately resistant highly resistant Proposed new wording: Resistance to Powdery mildew (Podosphaera	(Podosphaera xanthii) (Sf) sensibilité résistance moyenne forte résistance Résistance à l'oïdium (Podosphaera xanthii)	Echten Mehltau (Podosphaera xanthii) (Sf) anfällig mäßig resistent hochresistent Resistenz gegen Echten Mehltau (Podosphaera xanthii)	blanco (Podosphaera xanthii) (Sf) susceptible intermedia alta Resistencia al mildiú blanco (Podosphaera	Flamingo	2

hochresistent

alta

Cordoba

3

Current wording:

47.	Resistance to downy mildew	Résistance au mildiou (Pseudoperonospora	Resistenz gegen Falschen Mehltau	Resistencia al mildiú velloso del pepino		
(+)	(Pseudoperonospora cubensis) (Pc)	cubensis) (Pc)	(Pseudoperonospora cubensis) (Pc)	(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
40	Current wording:	Distriction and his	Basistana na na	Paristancia a la		
48. (+)	Resistance to Corynespora blight	Résistance à la	Resistenz gegen	Resistencia a la		
	and target leaf spot (Corynespora cassiicola) (Cca)	pourriture corynespora et à la septoriose (Corynespora cassiicola) (Cca)	Corynespora- Blattfleckenkrank-heit (Corynespora cassiicola) (Cca)	mancha foliar		
QL	and target leaf spot (<i>Corynespora</i>	et à la septoriose (Corynespora	Blattfleckenkrank-heit (Corynespora	mancha foliar (<i>Corynespora</i>	Cerrucho, Goya, Pepinova	1
QL	and target leaf spot (<i>Corynespora</i> <i>cassiicola</i>) (Cca)	et à la septoriose (Corynespora cassiicola) (Cca)	Blattfleckenkrank-heit (Corynespora cassiicola) (Cca)	mancha foliar (Corynespora cassiicola) (Cca)	Cerrucho, Goya, Pepinova Corona, Cumlaude, Edona	1 9
QL	and target leaf spot (Corynespora cassiicola) (Cca) absent	et à la septoriose (Corynespora cassiicola) (Cca) absente	Blattfleckenkrank-heit (Corynespora cassiicola) (Cca) fehlend	mancha foliar (Corynespora cassiicola) (Cca)		•
QL 48. (*) (+)	and target leaf spot (Corynespora cassiicola) (Cca) absent present	et à la septoriose (Corynespora cassiicola) (Cca) absente	Blattfleckenkrank-heit (Corynespora cassiicola) (Cca) fehlend vorhanden Resistenz gegen	mancha foliar (Corynespora cassiicola) (Cca)		•
48.	and target leaf spot (Corynespora cassiicola) (Cca) absent present Proposed new wording: Resistance to Corynespora blight and target leaf spot (Corynespora	et à la septoriose (Corynespora cassiicola) (Cca) absente présente Résistance à la pourriture corynespora et à la septoriose (Corynespora	Blattfleckenkrank-heit (Corynespora cassiicola) (Cca) fehlend vorhanden Resistenz gegen Corynespora- Blattfleckenkrankheit (Corynespora	mancha foliar (Corynespora cassiicola) (Cca) ausente presente Resistencia a la mancha foliar (Corynespora		•

Current wording:

49.	Resistance to Cucumber Vein	Résistance au virus du jaunissement des	Resistenz gegen Cucumber Vein	Resistencia al virus de las venas amarillas del		
(+)	Yellowing Virus (CVYV)	nervures du concombre	Yellowing Virus (CVYV)	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
50. (+)	Current wording: 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

<u>Proposal to Include a Revised Format for Disease Resistance Characteristics</u> (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 x 10⁵

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 daysFrom 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 2. Quarantine status 3. Host species 4. Source of inoculum 5. Isolate	Cladosporium cucumerinum No Cucumissativus (cucumber or gherkin) Naktuinbouw (NL)
Establishment isolate identity Establishment pathogenicity Multiplication inoculum	expected reactions on resistant standard varieties symptoms on susceptible standard varieties
8.1 Multiplication medium 8.2 Multiplication variety 8.3 Plant stage at inoculation	Agar medium e.g.:Potato Dextrose Agar (PDA)
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	nom 7 o days old suboditules in the dark at 20 o
8.8 Shelflife/viability inoculum	4 days at 4°C
9. Format of the test	4 days at 4 C
	at locat 20
9.1 Number of plants per genotype	at least 20
9.2 Number of replicates	Frantara Charubina Daninay CO (Cyanantible)
9.3 Control varieties	Frontera, Cherubino, Pepinex 69 (Susceptible)
A	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	Tioudi, comparativo
[1] Susceptible: Frontera	brown lesions on cotyledons and plant death
[9] Resistant: Corona	without symptoms, or with green lesions, or browning of the
[0] Noolotant. Oolona	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	QL
UPOV characteristic states	Q ∟
	tomporature and humidity
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

<u>Preparation of inoculum:</u> 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: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	1,
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,	light mottle; chlorotic islands
Gardon	
[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
44.40%	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	QN; [1] 3-4; [2] 5-6; [3] 7
UPOV characteristic states	4. Cumptoma will develop from ring and into recess (Marture)
13. Critical control points	1. Symptoms will develop from ring spot into mosaic (Ventura)

- 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 10⁵ 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. <u>Susceptible</u>: hypocotyls and cotyledons infected, first leaf strongly infected, high sporulation.

2. <u>Moderately resistant</u>: hypocotyls not infected, cotyledons and first leaf moderately infected with moderate sporulation, moderate colonization.

3. <u>Highly resistant</u>: 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)
6. Establishment isolate identity	expected reactions on resistant standard varieties
Establishment pathogenicity Multiplication inoculum	symptoms on susceptible standard varieties
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° spores/ml
8.8 Shelflife/viability inoculum	15 minutes
9. Format of the test	at least 20
9.1 Number of plants per genotype9.2 Number of replicates	at least 20
9.3 Control varieties	Ventura, Corona (Susceptible) Flamingo (IR);
3.5 Control varieties	Bella, Aramon, Cordoba (Resistant)
9.4 Test design	Bolia, Filamon, Gordoba (Feolotani)
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 1 st inoculation, first leaf at final inoculation
10.4 Inoculation method	Spraying,
10 E First shear estion	inoculation repeated on day 3, 5 and 6 after 1 st
10.5 First observation	10 dpi 14 dpi
10.7 Final observations	14 арі
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,	Symptoms on cotyledons are disregarded.
Cordoba:	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
10. Offical control politic	higher temperatures
	mg. o. tomporataroo

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:
 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 (<i>Pseudoperonospora cubensis</i>) No <i>Cucumissativus</i> (cucumber or gherkin)
Establishment isolate identity Establishment pathogenicity Multiplication inoculum	expected reactions on resistant standard varieties symptoms on susceptible standard varieties
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	at locat 20
9.1 Number of plants per genotype9.2 Number of replicates	at least 20
9.3 Control varieties	Pepinex 69, SMR 58 (susceptible), Poinsett 76 (intermediate
o.o Control variotico	resistant)
9.4 Test design	rosionarily
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	Diving a him a new detine leaves
10.1 Preparation inoculum	By washing sporulating leaves 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	OL [1] augaentible: [0] moderately and highly registant
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

10⁵ spores/ml. To be kept for a maximum of 4 days in a

refrigerator at 4°C

<u>Preparation of inoculum:</u> 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 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 daysFrom 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
Host species Source of inoculum	Cucumis sativus (cucumber or gherkin)
5. Isolate	Naktuinbouw (NL)
6. Establishment isolate identity	expected reactions on resistant standard varieties
7. Establishment pathogenicity	symptoms on susceptible standard varieties
8. Multiplication inoculum	symptoms on susceptible standard varieties
8.1 Multiplication medium	PDA at 20°C in darkness
8.2 Multiplication variety	1 Di Cat 20 O III dandiloso
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
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,
0.47	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
olo opoda mododico	closed day and night 3 dpi, closed only in night >3 dpi
10. Inoculation	orocoa day arra riigin o api, orocoa oriiy iir riigin yo api
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	Visual: comparative: mainly an actulation and first loof
11.1 Method	Visual; comparative; mainly on cotyledon and first leaf
[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
[1] 2, Gaddepublic Golfacile	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	QL
UPOV characteristic states	
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)

2. Quarantine status	1. Pathogen	Cucumber vein yellowing virus
4. Source of inoculum. 5. Isolate. 6. Establishment pathogenicity. 7. Establishment pathogenicity. 8. Multiplication medium. 8.1 Multiplication medium. 8.2 Multiplication medium. 8.3 Plant stage at inoculation. 8.4 Inoculation medium. 8.5 Inoculation medium. 8.6 Harvest of inoculum 8.7 Check of harvested inoculum 8.8 Shelflife/viability inoculum. 8.8 Shelflife/viability inoculum. 8.9 Format of the test 9.1 Number of plants per genotype. 9.2 Number of replicates 9.3 Control varieties. 7. Test design 9.5 Test facility. 9.6 Temperature. 9.6 Temperature. 9.7 Light. 9.8 Season. 9.9 Special measures. 10. Inoculation 10.1 Preparation inoculum. 10.3 Plant stage at inoculum. 10.3 Plant stage at inoculation. 10.1 Preparation inoculum. 10.4 Nonculation method. 10.5 Second observation. 10.5 Escond observation. 10.1 Second observation. 10.1 Susceptible 4. Ventura. 11. Susceptible 4. Ventura. 12. Resistant 5. Dina. 13. Resistant 5. Dina. 13. Preparation of data in terms of UPOV characteristic states 11.4 Off-types. 12. Interpretation of data in terms of UPOV characteristic states 12. Critical control points. 15. Incitical control points. 16. Second control points. 17. Staget and susceptible control inoculation susceptible control inoculation (cyledons yeighte variety (e.g. Korinda) (cyledons freeze-dried leaf (cyledons yeighte variety (e.g. Korinda) (cyledons yeighte as susceptible 4. Ventura (susceptible 3. Sorinda, Corona. 11 Susceptible 3. Korinda, Corona. 12 Susceptible 3. Korinda, Corona. 13 Resistant 5. Dina. 14 piff first leaf symptoms 15. Indication of test. 16 Second observation. 17 Second observation. 18 Second observation. 19 Resistant 5. Dina. 19 Resistant 5. Dina. 19 Resistant 5. Dina. 19 Resistant 6. Summerstar. 10 Standards should conform to description; describe if different. Variation within standard should not exceed 1 scale point varieties may have a slight discolorat		
5. Isolate e.g. KB18 6. Establishment isolate identity resistant and susceptible controls 7. Establishment pathogenicity susceptible control inoculation 8. Multiplication inoculum 8. Multiplication weldium leaf 8.2 Multiplication variety susceptible variety (e.g. Korinda) 8. Plant stage at inoculation 8.1 Plant stage at inoculation method green 8.5 Inoculation method green from to first leaf 8.6 Harvest of inoculum freeze-dried leaf 8.7 Check of harvested inoculum 8.7 Check of harvested inoculum 8.7 Check of harvested inoculum 8. Shefflife/viability inoculum 8. Shefflife/viability inoculum 8. Shefflife/viability inoculum 9. Format of the test 9.1 Number of plants per genotype at least 30 9.2 Number of replicates 9.3 Control varieties from the test 9.3 Control varieties from the test 9.3 Control varieties from the test 9.3 Parts tacility greenhouse 16-30°C 9. Emperature free free free free free free free f		,
6. Establishment isolate identity. 7. Establishment pathogenicity. 8. Multiplication inoculum 8.1 Multiplication medium. 8.2 Multiplication medium. 8.3 Plant stage at inoculation 8.5 Inoculation method. 8.6 Harvest of inoculum 8.7 Check of harvested inoculum 8.8 Shelflife/viability inoculum. 8.8 Shelflife/viability inoculum. 8.9 Format of the test 9.2 Number of plants per genotype. 9.3 Control varieties. Corona, Korinda, Ventura (Susceptible), Summerstar, Dina, Tornac (Resistant) 7 Check of varieties. Corona, Korinda, Ventura (Susceptible), Summerstar, Dina, Tornac (Resistant) 7 Check of Narvested inoculum 8.8 Shelflife/viability inoculum. 8.9 Format of the test 9.2 Number of plants per genotype. 9.3 Control varieties. Corona, Korinda, Ventura (Susceptible), Summerstar, Dina, Tornac (Resistant) 7 Corona, Korinda, Ventura (Susceptible), Summerstar, Dina, Tornac (Resistant) 7 Corona, Korinda, Ventura (Susceptible), Summerstar, Dina, Tornac (Resistant) 7 Corona, Korinda, Ventura (Susceptible), Summerstar, Dina, Tornac (Resistant) 8.5 Test facility. 9.6 Temperature. 16-30°C 9.7 Light. 16-30°C 12.000 lux suggested; keep glasshouse free of aphids 10. Inoculation 10.1 Preparation inoculum 10.2 Quantification inoculum 10.3 Plant stage at inoculation 10.4 Inoculation method. 10.4 Inoculation method. 10.5 Second observation. 10.6 Second observation. 11.1 Method. 11.2 Observations 11.1 Method. 11.2 Observations scale 11.1 Susceptible 4. Ventura. 12.1 Resistant 5. Dina. 13. Pristable scale (II) Susceptible 4. Ventura. 13. Resistant 5. Dina. 14. Off-types. 15. Critical control points. 16. Critical control points. 17. Critical control points.		
7. Establishment pathogenicity 8. Multiplication modulum 8.1 Multiplication medium 8.2 Multiplication variety 8.3 Plant stage at inoculation 8.5 Inoculation medium 8.6 Harvest of inoculum 8.6 Harvest of inoculum 8.7 Check of harvested inoculum 8.8 Shelflife/viability inoculum 9. Format of the test 9.1 Number of plants per genotype 9.2 Number of replicates 9.3 Control varieties 9.3 Control varieties 9.4 Test design 9.5 Test facility 9.6 Temperature 9.7 Establishment pathogenicity 9.8 Season 9.9 Special measures 9.1 Dinoculation 10.1 Preparation inoculum 10.2 Quantification inoculum 10.3 Plant stage at inoculation 10.4 Inoculation method 10.5 First observation 10.4 First observation 10.5 First observation 10.1 Prinal observation 10.1 Prinal observation 10.1 Pissa pathonuse 11.0 Sbeschalbe sale 11 Susceptible 4. Ventura 12 Resistant 5. Dina 13 Plantstage at inoculation 11.2 Observation of data in terms of UPOV characteristic states 13. Critical control points 14. Off-types 15. Inoculation order in the designation of the veins of the variety (e.g. Korinda) 2 susceptible variety (e.g. Korinda) 2 susceptible variety (e.g. Korinda) 2 susceptible 4 ventura 2 susceptible variety (e.g. Korinda) 2 susceptible 4 ventura busice cold PBS + carborundum 1 leaf 2 susceptible 4 ventura 2 susceptible 4 ventura 3 plantstage at inoculation 3 plantstage at inoculation 4 test 4°C or on ice 4 the 4°C or on ice 5 h at 4°C or on ice 5 h at 4°C or on ice 6 or on ice 7 corna, Korinda, Ventura (Susceptible), Summerstar, Dina, Tornac (Resistant) 1 susceptible 4. Ventura busice carborundum of suspensions of the prevent leaf damage 1 dest 30 2 dest results in Apr/May; Sep/Oct 1 suspensions free of aphids 1 dest 90°C 1 suspensions free of aphids 1 dest 90°C 1 suspensions free of aphids 1 leaf 1 susceptible 4 ventura busice carborundum off to prevent leaf damage 1 dest 30 2 dest 90°C 1 suspensions free of aphids 1 dest 9		
8. Multiplication inoculum 8.1 Multiplication medium 8.2 Multiplication variety 8.3 Plant stage at inoculation 8.5 Inoculation medium 8.6 Harvest of inoculum 8.7 Check of harvested inoculum 8.8 Shelfilife/viability inoculum 8.9 Shelfilife/viability inoculum 9.1 A Test design 9.2 Number of plants per genotype 9.3 Control varieties 9.3 Control varieties 9.4 Test design 9.5 Test facility 9.6 Temperature 9.7 Light 9.8 Season 9.9 Special measures 12.000 lux suggested; keep glasshouse free of aphids 10.1 Preparation inoculum 10.1 Preparation inoculum 10.3 Plant stage at inoculation 10.4 Inoculation method 10.5 First observation 10.5 First observation 10.1 Discondation 10.1 Discondation 10.2 Quantification inoculum 10.3 Plant stage at inoculation 10.4 Inoculation method 10.5 First observation 10.5 First observation 11.1 Wethod 11.2 Observations 11.1 Method 11.2 Ussceptible 4. Ventura 19.3 Resistant 5. Dina 19.3 Resistant 6. Summerstar 19.4 Nerver and the fact of the test 10.4 Off-types 10.4 Interpretation of data in terms of 10.4 UPOV characteristic states 11.4 Off-types 12. Interpretation of data in terms of 12. UPOV characteristic states 13. Critical control points 14 control points 15. First observation of the veins of		
8.1 Multiplication medium		susceptible control inoculation
8.2 Multiplication variety		
8.3 Plant stage at inoculation cotyledons / appearance of first leaf 8.4 Inoculation medium. 8.5 Inoculation medium. 8.6 Harvest of inoculum 8.7 Check of harvested inoculum 8.8 Shelflife/viability inoculum 8.9 Format of the test 9.1 Number of plants per genotype 9.2 Number of replicates 9.3 Control varieties. 9.3 Control varieties. 9.4 Test design 9.5 Test facility 9.5 Test facility 9.6 Temperature 16-30°C 9.7 Light 10.1 Preparation inoculum 10.1 Preparation inoculum 10.3 Plant stage at inoculation 10.3 Plant stage at inoculation 10.4 Inoculation method 10.5 First observation 10.6 Second observation 11.1 Method. 12.1 Observations 11.1 Method. 12.1 Susceptible 3. Korinda, Corona 13 Susceptible 3. Korinda, Corona 13 Susceptible 3. Korinda, Corona 15 Resistant 5. Dina 16 Resona 17 Resistant 5. Dina 18 Resistant 5. Dina 19 Resistant 5. Dina 19 Resistant 5. Ciritical control points 11.4 Off-types 12. Ciritical control points 12 Ciritical control points 14 Ciritical control points 15 cotyledons at least an icarchord page at least 30 15 cotyledons (reczedride leaf 17 Carona (Resistant) 18 hat 4°C or on ice 19 Resistant 5. Dina interperation in coulum 19 Resistant 5. Dina interperation in 0.03 M phosphate buffer + c		
8.4 Inoculation medium 8.5 Inoculation method 8.6 Harvest of inocullum 8.7 Check of harvested inoculum 8.8 Shelflife/viability inoculum 9. Format of the test 9.1 Number of plants per genotype 9.2 Number of replicates 9.3 Control varieties 9.3 Control varieties 9.4 Test design 9.5 Test facility 9.6 Temperature 9.7 Light 165 at least 9.8 Season 9.9 Special measures 10. Inoculation 10. 1 Preparation inoculum 10.3 Plant stage at inoculation 10.4 Inoculation method 10.5 First observation 10.7 Final observations 11. 1 Method 11. 2 Observations 11. 1 Method 11. 2 Observations 11. 1 Wethod 11. 2 Usuaceptible 12. Observations 13. Visual; comparative; mainly on first leaf 11. 2 Observation scale 19 Resistant 5. Dina 19 Resistant 5. Dina 19 Resistant 6. Summerstar 19 Resistant 7. Tornac 11. 3 Validation of test 12. Other control points 12. Clift of the test 13. Crifical control points 12. Clift of the test 13. Crifical control points 12. Clift of the test 13. Crifical control points 8 h at 4°C or on ice 8 h at 4°C or on ice 8 h at 4°C or on ice 9 h at least 30 2 h at least 30 2 h at least 30 2 corona, Korinda, Ventura (Susceptible), Summerstar, Dina, Tornac (Pesistant) 16 h at least 10. A least 10. Susceptible, Summerstar, Dina, Tornac (Pesistant) 16. A or on ice 9 h at 4°C o		
8.5 Inoculation method		, , , , , , , , , , , , , , , , , , , ,
8.6 Harvest of inoculum 8.7 Check of harvested inoculum 8.8 Shelfliffe/viability inoculum 9. Format of the test 9.1 Number of paints per genotype 9.2 Number of replicates 9.3 Control varieties 9.3 Control varieties 9.4 Test design 9.5 Test facility 9.6 Temperature 9.7 Light 16h at least 9.8 Season 9.9 Special measures 10. Inoculation 10.1 Preparation inoculum 10.3 Plant stage at inoculation 10.4 Inoculation method 10.5 First observation 10.4 Final observations 10.7 Final observations 11. Observations 11. Observations 11. Weithod 11. Susceptible 3. Korinda, Corona 13 Resistant 5. Dina 19 Resistant 5. Dina 19 Resistant 5. Dina 19 Resistant 5. Tornac 11. Validation of test 11. Off-types 12. Interpretation of data in terms of UPOv characteristic states 13. Critical control points 8 h at 4°C or on ice 8 h at 4°C or on ice 8 h at 4°C or on ice 9 h at least 30 2 h at least 30 2 h at least 30 2 telest 30 2 h corona, Korinda, Ventura (Susceptible), Summerstar, Dina, Tornac (Resistant) 11 least 30 2 h at least 30 2 telest 30 2 h corona, Korinda, Ventura (Susceptible), Summerstar, Dina, Tornac (Resistant) 11. Bat least 30 2 telest 30 2 telest 30 2 h corona, Korinda, Ventura (Susceptible), Summerstar, Dina, Tornac (Resistant) 11. Bat least 30 2 telest 30 2		
8.7 Check of harvested inoculum 8.8 Shelflife/viability inoculum 8.9 Format of the test 9.1 Number of plants per genotype 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 9.6 Temperature 9.7 Light 9.8 Season 9.8 Season 9.9 Special measures 10.1 noculation 10.1 Preparation inoculum 10.3 Plant stage at inoculation 10.4 Inoculation method 10.5 First observation 10.6 Second observation 10.7 Final observations 10.8 Deservations 11.1 Method 12.2 Observations 12.1 Observations 13.1 Susceptible 3. Korinda, Corona 13 Susceptible 3. Korinda, Corona 14 Susceptible 3. Korinda, Corona 15 Susceptible 4. Ventura 19 Resistant 5. Dina 19 Resistant 5. Dina 19 Resistant 5. Dina 19 Resistant 6. Summerstar 10.4 Off-types 11.4 Off-types 12. Interpretation of data in terms of UPOV characteristic states 13. Critical control points Test day Cor on ice at least 30 At 4°C or on ice at least 30 At 4°C or on ice at least 30 At 4°C or on ice at least 30 At 4 least 30 Corona, Korinda, Ventura (Susceptible), Summerstar, Dina, Tornac (Resistant) at least 30 At east as 4 corona (Resistant) At east as 30 At east 30 At east 30 At east as 4 corona (Arona) (Arona		· · · · · · · · · · · · · · · · · · ·
8.8 helflife/viability inoculum		freeze-dried leaf
9. Format of the test 9.1 Number of plants per genotype		01.4400
9.1 Number of replicates 9.2 Number of replicates 9.3 Control varieties		8 h at 4°C or on ice
9.2 Number of replicates 9.3 Control varieties		at least 20
9.3 Control varieties		at least 30
Tornac (Resistant) 9.4 Test design 9.5 Test facility		Carona Karinda Vantura (Suggestible) Summaratar Dina
9.4 Test design 9.5 Test facility	9.5 Control varieties	· · · · · · · · · · · · · · · · · · ·
9.5 Test facility	0.4 Toot design	Tomac (Resistant)
9.6 Temperature		graanhousa
9.7 Light		
9.8 Season		
9.9 Special measures		
10. Inoculation 10.1 Preparation inoculum		· · · · ·
10.1 Preparation inoculum		12.000 lax daggootoa, koop gladdhodoo hoo or aphiad
active charcoal 10.2 Quantification inoculum 10.3 Plant stage at inoculation		fresh leaf ground in 0.03 M phosphate buffer + carborundum +
10.2 Quantification inoculum 10.3 Plant stage at inoculation		
10.3 Plant stage at inoculation	10.2 Quantification inoculum	
10.4 Inoculation method		cotyledons
10.5 First observation		·
10.6 Second observation	10.5 First observation	
11. Observations 11.1 Method	10.6 Second observation	
11.1 Method	10.7 Final observations	21 dpi, first and second leaf symptoms
11.2 Observation scale [1] Susceptible 3. Korinda, Corona [1] Susceptible 4. Ventura	11. Observations	
 [1] Susceptible 3. Korinda, Corona [2] Resistant 5. Dina	11.1 Method	Visual; comparative; mainly on first leaf
[1] Susceptible 4. Ventura		
[9] Resistant 5. Dina	[1] Susceptible 3. Korinda, Corona	mosaic; clear border between yellow and green
[9] Resistant 6. Summerstar		
[9] Resistant 7. Tornac	[9] Resistant 5. Dina	
11.3 Validation of test		
Variation within standard should not exceed 1 scale point 11.4 Off-types		
 12. Interpretation of data in terms of	11.3 Validation of test	
 12. Interpretation of data in terms of	11.4 Off-types	·
13. Critical control points resistant varieties may have a slight discoloration of the veins of	12. Interpretation of data in terms of	QL; [1] 3-4, Susceptible; [9] 5-7, Resistant
	UPOV characteristic states	
older leaves	13. Critical control points	
		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:

Duration of test:

Mechanical, by rubbing of cotyledons
From inoculation to reading: 14 days

Number of plants tested:

Standard varieties:

At least 15 plants

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	nesh of unea leaf
8.8 Shelflife/viability inoculum	8 h at 4°C or on ice
9. Format of the test	on at 4 C of on ice
	at least 20
9.1 Number of plants per genotype	at least 30
9.2 Number of replicates 9.3 Control varieties	Corona Vantura Hilton (Cuacantible) Thunder Cummaratar
9.3 Control varieties	Corona, Ventura, Hilton (Susceptible), Thunder, Summerstar,
O 4 Test desire	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	QL: [1]4-5: Susceptible; [9] 6-7: Resistant
UPOV characteristic states	1 /12
13. Critical control points	Resistant varieties may have a slight discoloration of the veins
- r	of older leaves. Susceptible varieties have systemic mosaic
	symptoms.
	A 1

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]