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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

TECHNICAL WORKING PARTY FOR VEGETABLES

Forty-Fourth Session
Veliko Tarnovo, Bulgaria, July 5 to 9, 2010

PARTIAL REVISION OF THE TEST GUIDELINES FOR LETTUCE
(DOCUMENT TG/13/10)

Document prepared by experts from the Netherlands

Introduction

1. At its forty-third session, held in Beijing, from April 20 to 24, 2009, the Technical Working Party for Vegetables (TWV) decided to include "Lettuce (partial revision: *Bremia* resistance)" in the agenda of its forty-fourth session to be held in Veliko Tarnovo, Bulgaria, from July 5 to 9, 2010.

2. In addition to the partial revision concerning *Bremia* resistance, the inclusion of an additional characteristic "Resistance to *Nasonovia ribisnigri* biotype Nr: 0" is also proposed.

Partial Revision of Test Guidelines for Lettuce (TG/13/10)

3. It is proposed to revise Test Guidelines for Lettuce (document TG/13/10) as follows:

(1) Amendment of characteristic 39 in the Table of Characteristics, in particular:
- to include "Isolate Bl: 26" and Isolate Bl: 27,

- to change example varieties in order that they will be the same as the validated differentials that are available through the International *Bremia* Evaluation Board (IBEB),
- to modify the abbreviated pathogen codes by adding a space between the colon and the number (e.g. Bl:2 is changed to Bl: 2) in accordance with the decision of the ISF pathogen coding committee.

(see Annex I)

(2) Amendment of Ad. 39 in Chapter 8, in particular:
- to up-date the table of *Bremia* differentials and races, including Bl: 26 and Bl: 27,
- to delete the information on binary sextet codes, because this can be found in the literature,
- to reorganize the information about methods with particular attention to the observation records and interpretation,
- to delete the information about *Bremia* races that is better provided elsewhere.

(see Annex II)

(3) Addition of Characteristic 41 “Resistance to *Nasonovia ribisnigri* biotype Nr: 0”
(see Annex III)

(4) Addition of an explanation for Characteristic 41 in Chapter 8 Ad 41
(see Annex IV)

(5) Addition of one item to Chapter 9
(see Annex V)

4. The amendment of characteristic 39 and Addition of characteristic 41 will result in the publication of a partially revised Test Guidelines for Lettuce (document TG/13/10 Rev.).

5 The above proposal (paragraphs 3 and 4) for a partial revision of the Test Guidelines for Lettuce was circulated by Circular E-1271 of May 5, 2010 to the TWV for comments. In response to a comment received from the Community Plant Variety Office (CPVO) of the European Union, the example varieties for characteristic 41 “Resistance to *Nasonovia ribisnigr*i biotype Nr: 0” were amended (see Annex III).

[Annexes follow]

ANNEX I

Amendment of characteristic 39 in the Table of Characteristics

Proposed amendments are indicated by:

highlighting: proposed addition
~~strikethrough~~: proposed deletion

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
39. **(+)**	**VG**	**Resistance to downy mildew (*Bremia lactucae*)**	**Résistance au mildiou (*Bremia lactucae*)**	**Resistenz gegen Falschen Mehltau (*Bremia lactucae*)**	**Resistencia al mildiú (*Bremia lactucae*)**		
QL							
39.1	**(b) (c)**	**Isolate Bl: 2**	**Isolat Bl: 2**	**Isolat Bl: 2**	**Aislado Bl: 2**		
		absent	absente	fehlend	ausente	Cobham Green, ~~Hilde II~~, Green Towers	1
		present	présente	vorhanden	presente	Ninja	9
39.2	**(c)**	**Isolate Bl: 5**	**Isolat Bl: 5**	**Isolat Bl: 5**	**Aislado Bl: 5**		
		absent	absente	fehlend	ausente	Cobham Green, ~~Hilde II~~, Green Towers	1
		present	présente	vorhanden	presente	Sabine	9
39.3	**(c)**	**Isolate Bl: 7**	**Isolat Bl: 7**	**Isolat Bl: 7**	**Aislado Bl: 7**		
		absent	absente	fehlend	ausente	Cobham Green, ~~Hilde II~~, Green Towers	1
		present	présente	vorhanden	presente	Valmaine, ~~Verpia~~	9
39.4	**(c)**	**Isolate Bl: 12**	**Isolat Bl: 12**	**Isolat Bl: 12**	**Aislado Bl: 12**		
		absent	absente	fehlend	ausente	Cobham Green, ~~Hilde II~~, Green Towers	1
		present	présente	vorhanden	presente	~~Danilla, Geisha,~~ Dandie, UCDM12	9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
39.5	**(c)**	**Isolate Bl: 14**	**Isolat Bl: 14**	**Isolat Bl: 14**	**Aislado Bl: 14**		
		absent	absente	fehlend	ausente	Cobham Green, ~~Hilde~~, Green Towers	1
		present	présente	vorhanden	presente	~~Santis, Sifra, Verpia~~, Colorado, Ninja	9
39.6	**(c)**	**Isolate Bl: 15**	**Isolat Bl: 15**	**Isolat Bl: 15**	**Aislado Bl: 15**		
		absent	absente	fehlend	ausente	Cobham Green, ~~Hilde II~~, Green Towers	1
		present	présente	vorhanden	presente	~~Mirian~~,. Colorado, Sabine	9
39.7 (*)	**(c)**	**Isolate Bl: 16**	**Isolat Bl: 16**	**Isolat Bl: 16**	**Aislado Bl: 16**		
		absent	absente	fehlend	ausente	Cobham Green, Green Towers, ~~Hilde II~~	1
		present	présente	vorhanden	presente	Argelès, Ninja	9
39.8	**(c)**	**Isolate Bl: 17**	**Isolat Bl: 17**	**Isolat Bl: 17**	**Aislado Bl: 17**		
		absent	absente	fehlend	ausente	Cobham Green, Green Towers, ~~Hilde II~~	1
		present	présente	vorhanden	presente	Argelès, Ninja	9
39.9	**(c)**	**Isolate Bl: 18**	**Isolat Bl: 18**	**Isolat Bl: 18**	**Aislado Bl: 18**		
		absent	absente	fehlend	ausente	Cobham Green, Green Towers, ~~Hilde II~~	1
		present	présente	vorhanden	presente	Argelès, Ninja	9
39.10	**(c)**	**Isolate Bl: 20**	**Isolat Bl: 20**	**Isolat Bl: 20**	**Aislado Bl: 20**		
		absent	absente	fehlend	ausente	Cobham Green, Green Towers, ~~Hilde II~~	1
		present	présente	vorhanden	presente	Argelès, Ninja	9

		English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
39.11	**(c)**	**Isolate Bl: 21**	**Isolat Bl: 21**	**Isolat Bl: 21**	**Aislado Bl: 21**		
		absent	absente	fehlend	ausente	Cobham Green, Green Towers, ~~Hilde II~~	1
		present	présente	vorhanden	presente	Argelès, Colorado ~~Ninja~~	9
39.12	**(c)**	**Isolate B1: 22**	**Isolat Bl: 22**	**Isolat Bl: 22**	**Aislado Bl: 22**		
		absent	absente	fehlend	ausente	Cobham Green, Green Towers, ~~Hilde II~~	1
		present	présente	vorhanden	presente	Discovery, Ninja, ~~Coralis, Torpedo~~	9
39.13	**(c)**	**Isolate B1: 23**	**Isolat Bl: 23**	**Isolat Bl: 23**	**Aislado Bl: 23**		
		absent	absente	fehlend	ausente	Cobham Green, Green Towers, ~~Hilde II~~	1
		present	présente	vorhanden	presente	Colorado, Discovery, Ninja	9
39.14	**(c)**	**Isolate B1: 24**	**Isolat Bl: 24**	**Isolat Bl: 24**	**Aislado Bl: 24**		
		absent	absente	fehlend	ausente	Argelès, Colorado	1
		present	présente	vorhanden	presente	Dandie, UC DM14, PIVT 1309	9
39.15	**(c)**	**Isolate B1: 25**	**Isolat Bl: 25**	**Isolat Bl: 25**	**Aislado Bl: 25**		
		absent	absente	fehlend	ausente	Colorado, ~~Penlake~~ Pennlake	1
		present	présente	vorhanden	presente	~~Angela~~Argelès, Ninja	9
39.16	**(c)**	**Isolate B1: 26**	**Isolat Bl: 26**	**Isolat Bl: 26**	**Aislado Bl: 26**		
		absent	absente	fehlend	ausente	Colorado, Discovery	1
		present	présente	vorhanden	presente	Balesta, Bedford	9
39.17	**(c)**	**Isolate B1: 27**	**Isolat Bl: 27**	**Isolat Bl: 27**	**Aislado Bl: 27**		
		absent	absente	fehlend	ausente	Balesta, Green Towers	1
		present	présente	vorhanden	presente	Bedford	9

[Annex II follows]

ANNEX II

Amendment of Ad. 39 in Chapter 8,

The current wording is reproduced on pages 1 to 6.
The proposed new wording is indicated on pages 7 to 9.

Current wording:

Ad. 39: Resistance to downy mildew (*Bremia lactucae*)

Useful Dm-Genes

DUS examiners should test for Dm-genes of practical value which are directly involved in giving useful resistance in lettuce varieties, and obscure or irrelevant Dm-genes need not routinely be tested.

The currently useful Dm-genes are: 2, 3, 5/8, 6, 7, 11, 14, 16 and 18, as well as R17, R36, R37 and R38 factors. Only these should be tested on a routine basis.

Special Tests

Special tests may be required for Dml, Dm4, Dm15 and Dm10 (useful in the United States of America and Australia).

If breeders claim the presence of Dm-genes other than those mentioned above, then they should state in the Technical Questionnaires how the presence of these genes could be detected and, if appropriate, submit the relevant *Bremia* isolate to the testing centre to verify the claim. Special tests may be carried out for other Dm-genes if claimed by breeders as being appropriate for DUS examination.

Bremia Races

The following *Bremia* races should be used to determine whether a lettuce variety possesses the Dm-genes listed above: Bl:2, Bl:5, Bl:7, Bl:12, Bl:14, Bl:15, Bl:16, Bl:17, Bl:18, Bl:20, Bl:21, Bl:22, Bl:23, Bl:24 and Bl:25. For special discrimination between Dm 5/8 and Dm 7, Bl:7 is proposed.

These isolates possess a wide range of virulences. For details, please refer to relevant literature.

New Isolates

Additional isolates could be added to test for any useful new Dm-genes that might arise.

If new isolates of *Bremia* arise that can either detect novel Dm-genes in lettuce varieties or effectively replace an isolate listed above, then these isolates should be added to those listed.

Testing of *Bremia* Isolates

There are two centres, the “Station nationale d’essais de semences” (SNES) in France and the NAK Tuinbouw in the Netherlands, which would verify and test the isolates listed above and any new isolates that are used in routine tests. These centres should make these verified isolates available, against payment of prescribed fees, to the testing centres of other UPOV members.

The addresses of the centres are as follows:

Station nationale d’essais de semences (SNES)
Rue Georges Morel
B.P. 24
49071 Beaucouzé Cedex
France
Tél. : +33 (0) 2 41 22 58 00
Tlcp. : +33 (0) 2 41 22 58 01
Mél. : service.clients@geves.fr

NAK Tuinbouw
Sotaweg 20
P.O. Box 40
2370 AA Roelofarendsveen
Pays-Bas
Tél. : + 31 (0) 71 332 62 62
Tlcp. : + 31 (0) 71 332 63 63
Mél. : info@naktuinbouw.nl

Table of *Bremia* differentials:

		Variety	Cobham Green		Lednicky	UC DM2	Dandie	R4T57D	Valmaine	Sabine		LSE 57/15	UC DM10	Capitan	Hilde II	Pennlake	UC DM14		PIVT 1309	LSE /18	LS-102	Colorado	Ninja	Discovery		Argeles	Sextet code
	Dm nr/R nr		0		1	2	3	4	5/8	6		7	10	11	12	13	14		15	16	17	18/	36	37		38	
	Sextet nr				1	2	3	4	5	6		7	8	9	10	11	12		13	14	15	16	17	18		19	
	Sextet value				1	2	4	8	16	32		1	2	4	8	16	32		1	2	4	8	16	32		1	
IBEB code	Alias																										
Bl:1	NL 1		+		+	+	-	+	-	-		-	+	-	+	+	+		-	-	-	-	-	-		-	BL-A 11/58/00/00
Bl:2	NL 2		+		+	+	+	+	+	+		-	+	(-)	+	+	+		-	-	-	(-)	-	-		+	BL-A 63/58/00/01
Bl:3	NL 3		+		-	-	-	+	+	+		+	+	-	+	+	(+)		+	-	-	-	-	(-)		-	BL-A 56/59/01/00
Bl:4	NL 4		+		+	+	-	+	+	(-)		+	+	(-)	+	+	+		-	(-)	-	(-)	-	-		-	BL-A 27/59/00/00
Bl:5	NL 5		+		+	-	+	-	-	-		+	+	-	+	+	-		+	-	-	-	-	-		-	BL-A 05/27/01/00
Bl:6	NL 6		+		+	+	-	+	+	(-)		-	+	+	+	+	+		-	(-)	-	-	-	-		-	BL-A 27/62/00/00
Bl:7	NL 7		+		+	+	+	+	-	+		+	+	-	+	+	+		-	-	-	-	-	-		-	BL-A 47/59/00/00
Bl:10	NL 10		+		+	+	+	+	+	+		+	+	(-)	+	+	(+)		(-)	-	-	-	-	-		-	BL-A 63/59/00/00
Bl:11	NL 11		+		+	-	-	+	+	+		+	+	-	+	+	+		+	+	-	-	-	-		-	BL-A 57/59/03/00
Bl:12	NL 12		+		+	-	-	+	+	+		+	+	+	+	+	+		+	+	-	-	-	-		-	BL-A 57/63/03/00
Bl:13	NL 13		+		+	-	+	-	+	(-)		+	+	+	+	+	+		-	-	-	-	-	-		-	BL-A 21/63/00/00
Bl:14	NL 14		+		+	+	+	+	+	+		-	+	+	+	+	+		-	-	-	-	-	-		-	BL-A 63/62/00/00
Bl:15	NL 15		+		+	+	+	+	+	-		+	+	+	+	+	-		-	-	-	-	-	-		-	BL-A 31/31/00/00
Bl:16	NL 16/BL-16		+		+	+	+	+	+	+		+	+	+	+	+	-		-	+	-	-	-	-		-	BL-A 63/31/02/00
Bl:17	BL-17		+		-	+	+	-	+	-		+	+	-	+	+	+		+	-	-	+	-	+		-	BL-A 22/59/41/00
Bl:18	BL-18		+		+	+	-	+	+	+		+	+	+	+	+	-		-	+	-	+	-	-		-	BL-A 59/31/10/00
Bl:19	BL-19		+		+	+	+	+	+	+		-	+	+	+	+	+		-	-	-	-	-	-		+	BL-A 63/62/00/01

		Variety	Cobham Green		Lednicky	UC DM2	Dandie	R4T57D	Valmaine	Sabine		LSE 57/15	UC DM10	Capitan	Hilde II	Pennlake	UC DM14		PIVT 1309	LSE /18	LS-102	Colorado	Ninja	Discovery		Argeles	Sextet code
Bl:20	BL-20		+		+	+	+	+	+	+		+	+	+	+	+	-		-	+	-	+	-	-		-	BL-A 63/31/10/00
Bl:21	BL-21		+		+	+	+	+	+	+		+	+	+	+	+	-		+	+	-	-	+	+		-	BL-A 63/31/51/00
Bl:22	BL-22		+		+	+	-	+	+	+		+	+	+	+	+	+		+	-	-	+	-	-		-	BL-A 59/63/09/00
Bl:23	BL-23		+		+	+	+	+	+	+		+	+	+	+	+	-		-	+	-	-	-	-		+	BL-A 63/31/02/01
Bl:24	BL-24		+		+	+	-	+	+	+		+	+	+	+	+	-		-	+	-	+	-	-		+	BL-A 59/31/10/01
Bl:25	BL-25		+		+	+	-	+	+	+		+	+	+	+	+	-		-	+	-	+	-	+		-	BL-A 59/31/42/00
	S1		+		+	-	+	+	+	+		+	+	-	+	+	+		+	-	-	-	-	-		-	BL-A 61/59/01/00
	SF1		+		+	+	+	-	+	-		+	+	-	+	+	+		+	+	-	-	-	+		-	BL-A 23/59/35/00
	IL4		+		+	+	-	+	+	-		+	+	+	+	+	+		+	+	-	-	-	-		+	BL-A 27/63/03/01
	CS9		+		+	+	+	+	+	+		+	+	+	+	+	+		+	-	-	-	-	-		-	BL-A 63/63/01/00
	TV		+		+	+	+	+	+	+		+	+	-	+	+	+		+	+	-	-	-	-		-	BL-A 63/59/03/00

"+": susceptible
"-": resistant
"(-)": incomplete resistance
"(+)" incomplete susceptibility

Use of the sextets method to describe the resistance of varieties of lettuce to *Bremia*:

The resistance genes or Dm factors are grouped together in sixes (sextet):

1st sextet : 1, 2, 3, 4, 5/8, 6
2nd sextet : 7, 10, 11, 12, 13, 14
3rd sextet : 15, 16, 17, 18, 36, 37
4th sextet : 38

Each resistance gene or Dm factor receives a sextet number and each sextet number has a specific value (see table below).

Within each sextet, the values are allocated as follows:

- race overcoming the gene or Dm factor – (+) = sextet value
- race not overcoming the gene or Dm factor – (-) = 0 value.

All these values are then added together within the sextet in order to obtain an overall number per sextet. This number allows the race virulence spectrum to be found (only one virulence combination can correspond to a sextet value).

	1st sextet						2nd sextet						3rd sextet		
Dm	1	2	3	4	5/8	6	7	10	11	12	13	14	15	16	…
Sextet number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	…
Sextet value	1	2	4	8	16	32	1	2	4	8	16	32	1	2	…
Sextet value	+	+	-	+	-	-	-	+	-	+	+	+	-	-	
	1 + 2 + 0 + 8 + 0 + 0 = 11						0 + 2 + 0 + 8 + 16 + 32 = 58						0 + 0		…

Thus, a race with a maximum value of 63 for a sextet is virulent on all the genes or Dm and, conversely, a 0 value characterizes non-virulence on the six genes or Dm of the same sextet.

Resistance Testing Methods

The following guidelines are suggested for *Bremia* testing:

(a) Maintenance: *Bremia* races should be maintained on varieties possessing no known Dm-genes, or only obscure Dm-genes, e.g. Cobham Green, Lobjoits Green Cos, Hilde (Dm12), Olof. An alternative would be to use varieties/breeding lines which are selective for each particular isolate. The purity and quality of these maintenance varieties is important and it may be necessary to commission a seed producer to produce an adequate supply of good quality seed.

(b) Host differentials: Standard control varieties, that express the resistance genes that are being tested for, should always be used in tests, as a check. These standard varieties are available from GEVES Brion in France and NAK Tuinbouw, Netherlands:

GEVES Brion
Domaine de la Boisselière
49250 Brion
France

NAK Tuinbouw
Sotaweg 20, P.O. Box 40
2370 AA Roelofarendsveen
Netherlands

(c) Sample Size: At least 30 separate plants of each variety should be tested to establish the uniformity of the variety's Dm-gene component.

(d) Temperature: Incubation of inoculated seedlings or leaf discs should be at 15-18ºC.

(e) Inoculum Concentration: The optimum is around 1×10^5 spores per ml; at least 3×10^4 should be used. If inoculated seedlings are used, they may be inoculated prior to the emergence of the first leaf.

(f) Illumination: Adequate illumination should be provided for good plant growth. Seedlings should have fully expanded cotyledons and the plants should not be etiolated.

(g) Recording: The recording time should be as follows:

- First recording: when the control has maximum sporulation;
- Second recording: 3 days after first recording;
- Third recording: 3 days after second recording.

(In case of resistant varieties some plants may show leaf necrosis at the first recording.)

P*roposed new wording*

Ad. 39: Resistance to downy mildew (*Bremia lactucae*)

Availability of *Bremia* isolates and differentials

The "Station nationale d'essais de semences" (SNES) in France and Naktuinbouw in the Netherlands verify and test *Bremia* isolates as defined and denominated by the International *Bremia* Evaluation Board (IBEB). SNES and Naktuinbouw are responsible for delivery of denominated isolates to the testing centres of other UPOV members against payment of prescribed fees.

The addresses of the centres are as follows:

Station nationale d'essais de semences (SNES)
Rue Georges Morel
B.P. 24
49071 Beaucouzé Cedex
France
Tél. : +33 (0) 2 41 22 58 00
Tlcp. : +33 (0) 2 41 22 58 01
Mél. : service.clients@geves.fr

Naktuinbouw
Sotaweg 22
P.O. Box 40
2370 AA Roelofarendsveen
Pays-Bas
Tel. : + 31 (0) 71 332 62 62
Fax. : + 31 (0) 71 332 63 63
Email : info@naktuinbouw.nl

The common differential set of lettuce varieties and lines for determination of Bremia isolates is available from Naktuinbouw in The Netherlands (address as above) and GEVES in France at the following address:

GEVES Brion
Domaine de la Boisselière
49250 Brion
France

Resistance Testing Methods

(a) Maintenance: *Bremia* races may be maintained on varieties or breeding lines which are more or less selective for each particular isolate. It is essential to multiply Bl: 27 on selective plant material e.g. NunDm17.

(b) Host differentials: The host differential set that can distinguish all important *Bremia* races should always be used in tests, as a check on the identity of the isolate.

(c) Sample Size: minimum 30 plants

(d) Temperature: Incubation of inoculated seedlings or leaf discs should be at 15-18ºC.

(e) Inoculum Concentration: The optimum is around 1×10^5 spores per ml; at least 3×10^4 should be used.

(f) Illumination: Adequate illumination should be provided for good plant growth. Seedlings should have fully expanded cotyledons and the plants should not be etiolated.

(g) Recording: The recording time should be after 7, 10 and 13 days, or two of these three times. The time of maximum sporulation should occur in this period.

(h) Substrate: Seedling tests may be conducted on potting soil substrate or a substrate of paper wetted with a suitable mineral nutrient solution. Leaf disc tests may be conducted on wet paper without nutrients. Generally, the test on soil substrate will give a better discrimination of resistance and susceptibility.

(i) Observation and interpretation:

Table legend	Observation	Interpretation
+	Abundant or normal sporulation on cotyledons	Susceptible
(+)	Normal sporulation and necrotic spots	Susceptible
(-)	Necrosis and (sometimes) sparse sporulation on cotyledons	Resistant
-	no symptoms	Resistant

Table of *Bremia* differentials and races:

Isolates / Differentials	Green Towers	Lednicky	UC DM2	Dandie	R4T57D	Valmaine	Sabine	LSE 57/15	UC DM10	Capitan	Hilde II	Pennlake	UC DM14	NunDm15	LSE/18	NunDm17	Colorado	Ninja	Discovery	Argelès	RYZ 2164	RYZ 910457	Bedford	Balesta	Bellissimo
Bl: 1	+	+	+	-	+	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Bl: 2	+	+	+	+	+	+	+	-	+	(-)	+	+	+	-	-	-	(-)	-	-	+	-	-	-	+	+
Bl: 3	+	-	-	-	+	+	+	+	+	-	+	+	(+)	+	-	-	-	-	(-)	-	-	-	-	-	+
Bl: 4	+	+	+	-	+	+	(-)	+	+	(-)	+	+	+	-	(-)	-	(-)	-	-	-	-	-	-	-	-
Bl: 5	+	+	-	+	-	-	-	+	+	-	+	+	-	+	-	-	-	-	-	-	-	(-)	-	-	-
Bl: 6	+	+	+	-	+	+	(-)	-	+	+	+	+	+	-	-	-	-	-	-	-	-	(-)	-	-	-
Bl: 7	+	+	+	+	+	-	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Bl: 10	+	+	+	+	+	+	+	+	+	(-)	+	+	(+)	-	-	-	-	-	-	-	-	(-)	-	-	-
Bl: 11	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-					-
Bl: 12	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Bl: 13	+	+	-	+	-	+	(-)	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Bl: 14	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Bl: 15	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Bl: 16	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-
Bl: 17	+	-	+	+	-	+	-	+	+	-	+	+	+	+	-	-	+	-	+	-	-	-	(+)	-	-
Bl: 18	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-	-	-	-	-	-	-
Bl: 20	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-	-	-	-	-	-	-
Bl: 21	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	-	-	(-)	-	-
Bl: 22	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	(-)	+	-
Bl: 23	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	+	-	-	-	-	-
Bl: 24	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-	+	-	-	-	-	-
Bl: 25	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	-	+	-	-	-	-	-	-
Bl: 26	+	+	+	+	+	+	(+)	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-	-	-	-
Bl: 27	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	-	+	-

Note on table of Bremia differentials

The differential NunDm17 is a replacement for Ls102. The differential NunDm15 is a replacement for PIVT 1309. Ls102 and PIVT1309 have the same resistance pattern but poor germinability. More detailed information about the use of the table can be found in the relevant literature in chapter 9.

[Annex III follows]

ANNEX III

Addition of Characteristic 41 “Resistance to *Nasonovia ribisnigri* biotype Nr: 0”

Proposed wording:

41 **(+)**	**VG**	**Resistance to *Nasonovia ribisnigri* biotype Nr: 0**	**Résistance au *Nasonovia ribisnigri* biotype Nr: 0**	**Resistenz gegen *Nasonovia ribisnigri* Biotyp Nr: 0**	**Resistencia al *Nasonovia ribisnigri* biotype Nr: 0**		
		absent	absente	fehlend	ausente	Green Towers, Abel, Nadine	1
		present	présente	vorhanden	presente	Silvinas, Barcelona, Dynamite	9

(In the above table, the yellow-highlighted example varieties were added in response to the comment received from the Community Plant Variety Office (CPVO) of the European Union.)

Comment received from CPVO:

In 2009 the CPVO partially revised its lettuce protocol and the EU vegetable experts agreed to add a new non-asterisked disease resistance characteristic, “Resistance to *Nasonovia ribisnigri*”. However, in the CPVO protocol the characteristic covered the whole *N. ribisnigri* pathogen species, whereas this proposal for the UPOV guideline revision is limited to the biotype Nr: 0. In addition, the example varieties quoted in the CPVO protocol for this disease resistance are completely different to the ones proposed in the partial revision to the UPOV guideline for *N. ribisnigri* biotype Nr: 0.

Answer and proposal:

In 2007 a new variant of the *Nasonovia ribisnigri* aphid has been identified. Research at Naktuinbouw has confirmed that this variant has overcome the resistance in most varieties. This was communicated through a press release and published in several places, for example in the Dutch weekly “Groenten en Fruit” in 2008, volume 19, page 29 and also on the internet on **http://priorartdatabase.com/IPCOM/000176078**. Several companies have immediately reacted to this news and are already adding the biotype number to the name of the organism. The variant aphids are now still present in most of the major lettuce cropping areas in North West Europe. For these reasons it is proposed to add the biotype designation 0 to the species name *Nasonovia ribisnigri*.

There is no special reason for changing the example varieties. To the best of knowledge all the mentioned Nr:0 resistant varieties are interchangeable (and also the susceptible varieties are interchangeable). Varieties with resistance to the new biotype Nr:1 have not yet been developed. It is proposed to add the example varieties of the CPVO protocol to the revision of the UPOV guideline. This is done and highlighted in yellow in the characteristic above.

[Annex IV follows]

ANNEX IV

Addition of an explanation in Chapter 8 Ad. 41

Proposed wording:

Ad. 41: Resistance to *Nasonovia ribisnigri* biotype Nr: 0

Maintenance of biotype

Nasonovia ribisnigri is a leaf aphid and may be maintained alive on susceptible lettuce plants in aphid-proof chambers or tents in a glasshouse. *N. ribisnigri* is usually green, but some biotypes are red. A red aphid is easier to see on a green leaf. Therefore red biotypes are usually preferable. The aphid's body size is 1.5-2.5 mm. The body has 7 dark spots. The ends of the legs are black.

The common biotype Nr: 0 can be distinguished from resistance-breaking biotypes by means of a biotest using a suitable resistant control variety, for example Silvinas.

Multiplication:
On a susceptible variety at 20-22°C for 10-14 days. Aphids are shaken off into a Petri-dish.

Sowing:
12°C for germination and early growth; plant distance at least 5 cm.
Number of plants to be tested: 28.

Inoculation method:
Careful transfer of 5 aphids per plant using a fine paintbrush.
Plant stage at inoculation: 15 days.
Temperature: 20-22°C.

Observation:
First observation: 10 days after inoculation.
Second observation: daily check whether newborn aphids are mature (= red).
End of test: max. 15 days after inoculation.

Observation at end of test: Count the number of mature (= red) aphids on each plant.

Scale for observations:		Interpretation of data
0	no aphids	Resistant
1	1-5 aphids per plant	Resistant
2	6-10 aphids per plant	Undecided
3	> 10 aphids per plant	Susceptible

Remarks
Resistant control varieties and susceptible control varieties should have at least 95% (26/28) resistant plants and susceptible plants, respectively.
If more than 2 of 28 plants of the control varieties are undecided or off type, the experiment should be repeated.

[Annex V follows]

ANNEX V

Addition of one item to Chapter 9

The following literature should be added to Chapter 9:

"Van der Arend et al., 2007: Identification and nomination of new races of *Bremia lactucae* in Europe by IBEB until 2006. Eucarpia Leafy Vegetables 2007 Conference Abstracts, 18-20 April 2007, University of Warwick, Poster presentations, pp. 27 v.v."

[End of Annex V and of document]