ANNEX I

TEST GUIDELINES FOR LETTUCE: TG/13/11 REV.2
ADDITIONAL CHARACTERISTIC

Submitting Authority: CPVO (QZ)

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		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo **[iii]**	Note/ Nota
New 1.	**VG**	**Resistance to *Bremia lactucae* (Bl) Isolate PT2036**	**Résistance à *Bremia lactucae* (Bl) Isolat PT2036**	**Resistenz gegen *Bremia lactucae* (Bl) Isolat PT2036**	**Resistencia a *Bremia lactucae* (Bl) Aislado PT2036**		
QL		absent	absente	fehlend	ausente	Green Towers, Odra	1
		present	présente	vorhanden	presente	Templin	9

[i] indicate type of expression (QL, PQ, QN)
[ii] indicate method of observation (VG, VS, MG, MS)
[iii] example varieties to be provided for at least 2 states.

Explanation / Illustration (including extent of the use of the characteristic(s)):

Resistance to Bremia lactucae (Bl) isolate PT2036

1. Pathogen	*Bremia lactucae*
2. Quarantine status	no
3. Host species	lettuce - Lactuca sativa L.
4. Source of inoculum	Naktuinbouw (resistentie@naktuinbouw.nl)
5. Isolate	PT2036
6. Establishment isolate identity	test on differentials (see table below)
7. Establishment pathogenicity	test on susceptible varieties
8. Multiplication inoculum	
8.1 Multiplication medium	lettuce plantlets
8.2 Multiplication variety	susceptible variety, for example Green Towers.
8.3 Plant stage at inoculation	cotyledon to first leaf
8.4 Inoculation medium	tap water
8.5 Inoculation method	spraying a spore suspension
8.6 Harvest of inoculum	washing off from leaves
8.7 Check of harvested inoculum	counting spores
8.8 Shelf life/viability inoculum	2 hours at room temperature; 2 days in fridge
9. Format of the test	
9.1 Number of plants per genotype	at least 20
9.2 Number of replicates	-
9.3 Control varieties (informative)	differentials (see table below)
9.4 Test design	-
9.5 Test facility	climate room
9.6 Temperature	15°C-18°C
9.7 Light	adequate for good plant growth; seedlings should not etiolate. option: reduced light 24 hours after inoculation
9.8 Season	-
9.9 Special measures	plants may grow on wet blotting paper with or without a nutrient solution, on sand, or on potting soil (see point 13).

high humidity (>90%) is essential for infection and sporulation.

10. Inoculation

10.1 Preparation inoculum — washing off from leaves by vigorous shaking in a closed container

10.2 Quantification inoculum — counting spores; spore density should be 3.10^4-1.10^5

10.3 Plant stage at inoculation — cotyledon stage

10.4 Inoculation method — spraying till run-off
option: reduced light 24 hours after inoculation

10.5 First observation — beginning of sporulation on susceptible varieties (around 7 days after inoculation)

10.6 Second observation — 3-4 days after first observation (around 10 days after inoculation)

10.7 Final observations — 14 days after inoculation
two of these three observations may be sufficient; the third notation is optional for observation of evolution of symptoms in case of doubt. The day of maximum sporulation should occur in this period.

11. Observations

11.1 Method — visual observation of sporulation and necrotic reaction to infection

0.2 Observation scale — resistant

0 no sporulation, no necrosis
1 no sporulation, necrosis present
2 weak sporulation (much less than susceptible control) with necrosis
3 weak sporulation (less than susceptible control and not evolving between second and third observations) with necrosis
4 very sparse sporulation (not evolving between second and third observation) without necrosis
susceptible
5 reduced sporulation (compared to susceptible control) without necrosis
6 normal sporulation without necrosis

11.3 Validation of test — on standards.
In case of normal sporulation (same level as susceptible control) with necrosis, another test on bigger plants or other substrate must be undertaken.

12. Interpretation of data in terms of UPOV characteristic states — class 0, 1, 2, 3 and 4: resistant
class 5 and 6: susceptible

13. Critical control points — Reaction of standards (the infection pressure may vary between experiments, leading to slight differences in sporulation intensity); when the reactions are not clear the experiment should be repeated.
The sowing on soil can be used to see necrosis, but weak sporulation (much less than susceptible control) can appear; when testing on sand, spores can be confused with grains of sand.
In case of use of nutritive solution on blotting paper, a fungicide can be added to avoid contamination by saprophytes

	GreenTowers	Dandie	R4T57D	UC Dm14	NunDm15	CGDm16	Colorado	FrRsal-1	Argelès	RYZ 2164	RYZ910457	Bedford	Balesta	Bartoli	Design	Kibrille	Fenston	Bataille	RYZ20007	Set	D sextet code
		Dm3	Dm4	Dm14	Dm15	Dm16	Dm18	Rsal-1	R38												
ID	0	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18		
Sextet value		1	2	4	8	16	32	1	2	4	8	16	32	1	2	4	8	16	32		
PT2036	+	-	+	+	+	-	+	+	+	+	+	-	-	-	+	+	-	-	+	D	46-15-38

[Annex II follows]