E

TG/6/5(proj.2)
ORIGINAL: English
DATE: 2004-06-10

UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

DRAFT

LUCERNE *

UPOV code: MEDIC_SAT

(Medicago sativa L.and
Medicago x *varia* Martyn*)*

GUIDELINES

FOR THE CONDUCT OF TESTS

FOR DISTINCTNESS, UNIFORMITY AND STABILITY

prepared by experts from France

to be considered by the
Technical Working Party for Agricultural Crops at its thirty-third session,
to be held in Poznań, Poland, from June 28 to July 2, 2004

Alternative Names:*

Botanical name	*English*	*French*	*German*	*Spanish*
Medicago sativa L.	Lucerne, Alfalfa	Luzerne	Blaue Luzerne	Alfalfa, Mielga
Medicago x *varia* Martyn				

The purpose of these guidelines ("Test Guidelines") is to elaborate the principles contained in the General Introduction (document TG/1/3), and its associated TGP documents, into detailed practical guidance for the harmonized examination of distinctness, uniformity and stability (DUS) and, in particular, to identify appropriate characteristics for the examination of DUS and production of harmonized variety descriptions.

ASSOCIATED DOCUMENTS

These guidelines("Test Guidelines") should be read in conjunction with document TG/1/3, "General Introduction to the Examination of Distinctness, Uniformity and Stability and the Development of Harmonized Descriptions of New Varieties of Plants" (hereinafter referred to as the "General Introduction") and its associated "TGP" documents.

Other associated UPOV documents:

* These names were correct at the time of the introduction of these Test Guidelines but may be revised or updated. [Readers are advised to consult the UPOV Code, which can be found on the UPOV Website (www.upov.int), for the latest information.]

TABLE OF CONTENTS PAGE

1. Subject of these Test Guidelines

These Test Guidelines apply to all varieties of *Medicago sativa* L. and *Medicago* x *varia* Martyn.

2. Material Required

2.1 The competent authorities decide on the quantity and quality of the plant material required for testing the variety and when and where it is to be delivered. Applicants submitting material from a State other than that in which the testing takes place must ensure that all customs formalities and phytosanitary requirements are complied with.

2.2 The material is to be supplied in the form of seed.

2.3 The minimum quantity of plant material, to be supplied by the applicant, should be:

1 kilogram

The seed should meet the minimum requirements for germination, species and analytical purity, health and moisture content, specified by the competent authority. In cases where the seed is to be stored, the germination capacity should be as high as possible and should, be stated by the applicant.

2.4 The plant material supplied should be visibly healthy, not lacking in vigor, nor affected by any important pest or disease.

2.5 The plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If it has been treated, full details of the treatment must be given.

3. Method of Examination

3.1 Number of Growing Cycles

The minimum duration of tests should normally be two independent growing cycles.

3.2 Testing Place

Tests are normally conducted at one place. In the case of tests conducted at more than one place, guidance is provided in TGP/9 “Examining Distinctness”.

3.3 Conditions for Conducting the Examination

The tests should be carried out under conditions ensuring satisfactory growth for the expression of the relevant characteristics of the variety and for the conduct of the examination.

The optimum stage of development for the assessment of each characteristic is indicated in the second column of the Table of Characteristics.

The recommended method of observing the characteristic is indicated by the following key in the second column of the Table of Characteristics:

MG: single measurement of a group of plants or parts of plants
MS: measurement of a number of individual plants or parts of plants
VG: visual assessment by a single observation of a group of plants or parts of plants
VS: visual assessment by observation of individual plants or parts of plants

The recommended type of plot in which to observe the characteristic is indicated by the following key in the second column of the Table of Characteristics:

A: spaced plants
B: row plot
C: special test

3.4 Test Design

3.4.1 Each test should be designed to result in a total of at least 60 spaced plants and 10 meters of row plot, which should be divided between 3 replicates.

3.4.2 The design of the tests should be such that plants or parts of plants may be removed for measurement or counting without prejudice to the observations which must be made up to the end of the growing cycle.

3.5 Number of Plants / Parts of Plants to be Examined

3.5.1 Unless otherwise indicated, all observations on single spaced plants should be made on 60 plants or parts taken from each of 60 plants in the spaced plants plots.

3.5.2 Unless otherwise indicated, all measurements should be made on a total of 18 plants or parts of plants from the row plots, 6 taken from each of the 3 replicates.

3.6 Additional Tests

Additional tests, for examining relevant characteristics, may be established.

4. Assessment of Distinctness, Uniformity and Stability

4.1 Distinctness

4.1.1 General Recommendations

It is of particular importance for users of these Test Guidelines to consult the General Introduction prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in these Test Guidelines.

4.1.2 Consistent Differences

The differences observed between varieties may be so clear that more than one growing cycle is not necessary. In addition, in some circumstances, the influence of the environment

is not such that more than a single growing cycle is required to provide assurance that the differences observed between varieties are sufficiently consistent. One means of ensuring that a difference in a characteristic, observed in a growing trial, is sufficiently consistent is to examine the characteristic in at least two independent growing cycles.

4.1.3 Clear Differences

Determining whether a difference between two varieties is clear depends on many factors, and should consider, in particular, the type of expression of the characteristic being examined, i.e. whether it is expressed in a qualitative, quantitative, or pseudo-qualitative manner. Therefore, it is important that users of these Test Guidelines are familiar with the recommendations contained in the General Introduction prior to making decisions regarding distinctness.

4.2 Uniformity

4.2.1 It is of particular importance for users of these Test Guidelines to consult the General Introduction prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in these Test Guidelines:

4.2.2 The assessment of uniformity should be according to the recommendations for cross-pollinated varieties in the General Introduction.

4.3 Stability

4.3.1 In practice, it is not usual to perform tests of stability that produce results as certain as those of the testing of distinctness and uniformity. However, experience has demonstrated that, for many types of variety, when a variety has been shown to be uniform, it can also be considered to be stable.

4.3.2 Where appropriate, or in cases of doubt, stability may be tested, either by growing a further generation, or by testing a new seed stock to ensure that it exhibits the same characteristics as those shown by the previous material supplied.

5. Grouping of Varieties and Organization of the Growing Trial

5.1 The selection of varieties of common knowledge to be grown in the trial with the candidate varieties and the way in which these varieties are divided into groups to facilitate the assessment of distinctness are aided by the use of grouping characteristics.

5.2 Grouping characteristics are those in which the documented states of expression, even where produced at different locations, can be used, either individually or in combination with other such characteristics: (a) to select varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness; and (b) to organize the growing trial so that similar varieties are grouped together.

5.3 The following have been agreed as useful grouping characteristics:

Plant: tendency to grow during winter (fall dormancy) (characteristic 16)

5.4 Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the General Introduction.

6. Introduction to the Table of Characteristics

6.1 Categories of Characteristics

6.1.1 Standard Test Guidelines Characteristics

Standard Test Guidelines characteristics are those which are approved by UPOV for examination of DUS and from which members of the Union can select those suitable for their particular circumstances.

6.1.2 Asterisked Characteristics

Asterisked characteristics (denoted by *) are those included in the Test Guidelines which are important for the international harmonization of variety descriptions and should always be examined for DUS and included in the variety description by all members of the Union, except when the state of expression of a preceding characteristic or regional environmental conditions render this inappropriate.

6.2 States of Expression and Corresponding Notes

States of expression are given for each characteristic to define the characteristic and to harmonize descriptions. Each state of expression is allocated a corresponding numerical note for ease of recording of data and for the production and exchange of the description.

6.3 Types of Expression

An explanation of the types of expression of characteristics (qualitative, quantitative and pseudo-qualitative) is provided in the General Introduction.

6.4 Example Varieties

Where appropriate, example varieties are provided to clarify the states of expression of each characteristic.

6.5 Legend

(*) Asterisked characteristic – see Chapter 6 (Section 6.1.2)

QL Qualitative characteristic – see Chapter 6 (Section 6.3)
QN Quantitative characteristic – see Chapter 6 (Section 6.3)
PQ Pseudo-qualitative characteristic see Chapter 6 (Section 6.3)

MG: single measurement of a group of plants or parts of plants – see Section 3.3
MS: measurement of a number of individual plants or parts of plants – see Section 3.3
VG: visual assessment by a single observation of a group of plants or parts of plants – see Section 3.3
VS: visual assessment by observation of individual plants or parts of plants – see Section 3.3

(a)-(b) See Explanations on the Table of Characteristics in Chapter 8.1

(+) See Explanations on the Table of Characteristics in Chapter 8.

7. Table of Characteristics/Tableau des caractères/Merkmalstabelle/Tabla de caracteres

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
1.	**B**	**Plant: growth habit in autumn of the first year**					
QL		erect				KH Maraton, Körös 1	1
		semi erect				Jozso	3
		medium				Kakai legelö	5
		semi prostrate				Szentesi rona	7
		prostrate					9
2. (*)	**MS A MG B**	**Plant: natural height 2 weeks after the first autumn equinox following sowing (cut 2 weeks before equinox)**					
QN	**(a)**	short				Likarlu, Luzelle	3
		medium				Andela, Fauna	5
		tall				Magali	7
3. (*)	**MG B**	**Plant: natural height 6 weeks after the first autumn equinox following sowing (cut 2 weeks after equinox)**					
QN	**(a)**	short				Boja	3
		medium				Diane	5
		tall				Medalfa	7

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
4. **(+)**	**MS** **A** **MG** **B**	**Plant: natural height in spring (1 month after beginning of growing the year after sowing)**					
QN	**(a)**	short				Likarlu, Vertus	3
		medium				Diane, Rival	5
		tall				Letizia, Magali	7
5. **(*)** **(+)**	**MS** **A** **MG** **B**	**Time of beginning of flowering**					
QN		early				Alize	3
		medium				Luzelle	5
		late				Likarlu	7
6. **(*)**	**VS** **A**	**Flower:frequency of plants with very dark blue violet flowers**					
QN	**(b)**	absent or very low				Diane	1
		low				Sanditi	3
		medium				Andela	5
		high				Orca	7
7. **(*)**	**VS** **A**	**Flower: frequency of plants with variegated flowers**					
QN	**(b)**	absent or very low				Symphonie	1
		low				Luzelle, Letizia	3
		medium				Franken Neu, Likarlu	5
		high					7

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
8. (*)	**VS A**	**Flower:frequency of plants with cream, white or yellow flowers**					
QN	**(b)**	absent or very low				Europe	1
		low					3
		medium				Likarlu	5
		high					7
9. (*)	**MS A**	**Stem: length of the longest stem at full flowering (head included; when fully expanded)**					
QN		short				Likarlu	3
		medium				Franken Neu, Carmen	5
		long				Fauna	7
10. (+)	**MS A MG B**	**Plant: natural height 3 weeks after 1st cut**					
QN		short				Likarlu	3
		medium				Andela, Symphonie	5
		tall				Zenith	7
11. (+)	**MS A MG B**	**Plant: natural height 3 weeks after 2nd cut**					
QN		short				Likarlu	3
		medium				Franken Neu, Andela	5
		tall				Zenith	7

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
12. **(+)**	**MS A** **MG B**	**Plant: natural height 3 weeks after 3rd cut**					
QN		short				Likarlu	3
		medium				Timbale	5
		tall				Letizia, Zenith	7
13. **(+)**	**MS A**	**Plant: natural height 3 weeks after 4th cut**					
QN		short				Likarlu	3
		medium				Symphonie, Andela	5
		tall				Carmen, Zenith	7
14.	**MG B**	**Plant: natural height 2 weeks after the second autumn equinox following sowing (cut 2 weeks before equinox)**					
QN	**(a)**	short				Gibraltar	3
		medium				Fauna	5
		tall				Zenith	7
15.	**MG B**	**Plant: natural height 6 weeks after the second autumn equinox following sowing (cut 2 weeks after equinox)**					
QN	**(a)**	short				Boja	3
		medium				Europe	5
		tall				Zenith	7

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
16. **(+)**	**MG C**	**Plant: tendency to grow during winter**					
QN		Dormancy rating 1				Maverick	1
		Dormancy rating 2				Vernal	2
		Dormancy rating 3				Boja, Ranger	3
		Dormancy rating 4				Legend, Mercedes	4
		Dormancy rating 5				Archer	5
		Dormancy rating 6				Abi 700, Dorine	6
		Dormancy rating 7				Sutter, Oro	7
		Dormancy rating 8				Maricopa, Carmen	8
		Dormancy rating 9				CUF 101, Medina	9
		Dormancy rating 10				UC-1887	10
		Dormancy rating 11				UC-1465	11
17. **(+)**	**VS C**	**Resistance to *Verticillium albo-atrum***					
QN		low				Medalfa	3
		medium				Europe, Derby	5
		high				Vertus	7
18. **(+)**	**VS C**	**Resistance to *Ditylenchus dipsaci***					
QN		very low					1
		low				Europe	3
		medium					5
		high				Vertus	7

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
19.		**Resistance to *Colletotrichum trifolii***					
(+)							
QN		very low				Saranac	1
		low				Venus	3
		medium					5
		high				Saranac AR	7
		very high				Arc	9
20.		**Resistance to *Phytophtora medicaginis***					
(+)							
QN		very low				Hunterfield	1
		low					3
		medium				Trifecta	5
		high					7
		very high				Aquarius	9
21.		**Resistance to *Acyrthosiphon kondoi***					
(+)							
QN		very low				Hunter River	1
		low					3
		medium				Siriver	5
		high					7
		very high				Aurora	9

Char. No.	Method of Examination	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
22.		**Resistance to *Therioaphis maeulata***					
(+)							
QN		very low				Hunter River	1
		low					3
		medium				Trifecta	5
		high					7
		very high				Aurora	9

8. Explanations on the Table of Characteristics

8.1 Explanations covering several characteristics

Characteristics containing the following key in the second column of the Table of Characteristics should be examined as indicated below:

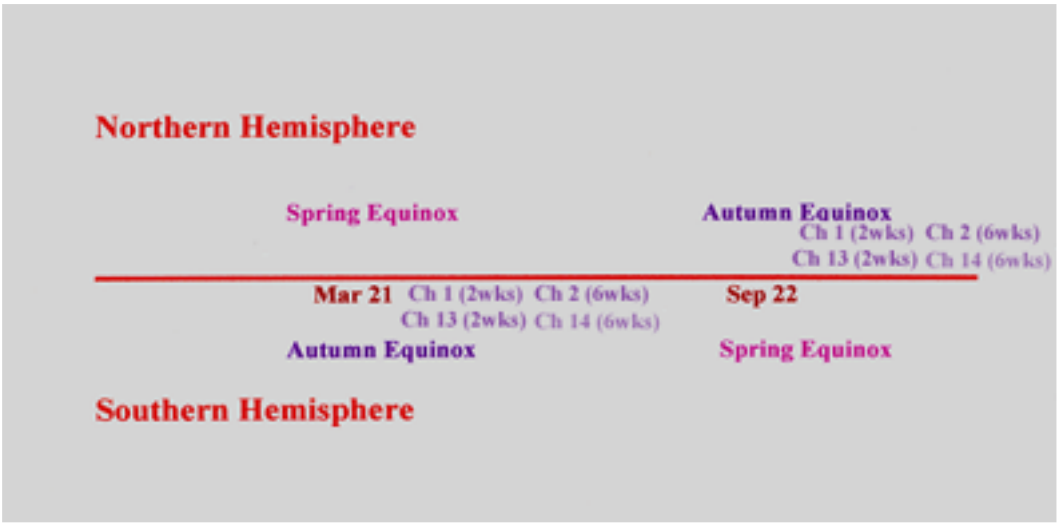
(a) Explanation for the characteristics 2, 3, 4, 13 and 14

The equinox referred to in characteristics 2, 3, 4, 13 and 14 and elsewhere in the text refers to the autumnal or fall equinox. This occurs on September 22 for the Northern hemisphere and March 21 for the Southern hemisphere. It is an appropriate date on which to base the plant height measurements relating to the degree of fall dormancy (which in reverse is called "winter-activity" in many countries in the southern hemisphere).

In Characteristics 1 and 2, the plant height measurements should be respectively taken 2 and 6 weeks after the first autumnal equinox.

In Characteristics 13 and 14, the plant height measurements should be respectively taken 2 and 6 weeks after the second autumnal equinox.

The following diagram shows the time of the year when these measurements should be taken for each of the hemispheres.



(b) Observations on flower color should be made at the beginning of flowering. The frequency should be assessed on spaced plants (VS). The states of expression cover the whole range from 1% to 100% although example varieties are so far not yet known for the whole range. Variegation is defined by the presence of yellow and violet pigments within the same flower. This combination may lead to the appearance of green color.

8.2 Explanations for individual characteristics

Ad. 4: Plant: natural height in spring (1 month after beginning of growing the year after sowing)

The measurement should be done one month after the earliest varieties start to grow and reach about 15 cm height.

Ad. 5: Time of beginning of flowering

A. The date of beginning of flowering of each single plant should be assessed. A single plant is considered to have headed when three inflorescences can be seen. From the single plant data a mean date per plot and a mean date per variety are obtained.

B. The date of beginning of flowering of row plots should be assessed. Row plots are considered to have headed when ¼ of inflorescence per plot can be seen. From the row plant data, a mean date per plot and a mean date per variety are obtained.

Ad. 10: Plant: natural height 3 weeks after 1st cut

The first cut should be done just after full flowering, when Characteristic 8: "Stem: Length of longest stem at full flowering (head included; when fully expanded)" has been assessed.

Ad. 11: Plant: natural height 3 weeks after 2nd cut

The plants should be cut just after the preceding characteristic (Plant, natural height 3 weeks after 1st cut) has been measured.

Ad. 12: Plant: natural height 3 weeks after 3rd cut

The plants should be cut just after the preceding characteristic (Plant, natural height 3 weeks after 2nd cut) has been measured.

Ad. 13: Plant: natural height 3 weeks after 4th cut

The plants should be cut just after the preceding characteristic (Plant, natural height 3 weeks after 3rd cut) has been measured.

Ad. 16: Plant: tendency to grow during winter.

This characteristic is consistent with the fall dormancy ratings, used to characterized Lucerne varieties. The notes could be compared to those already used for the fall dormancy rate. In reverse, this characteristic is called winter activity in many countries in the southern hemisphere. All varieties do not respond equally in the autumn to change in photoperiod and temperature and this is a strongly expressed genetic trait.

Growth should occur during the autumn period, but before a severe frost and/or in the beginning of spring. Local experience will provide information on which cut date provides the greatest separation among varieties for fall dormancy (Teuber *et al*., 1998; Montegano *et al*, 2002).

The tendency to grow during winter is assessed by several measurements of the height of a group of plants (MG). The following characteristics are used:

Char.1: Plant: natural height 2 weeks after the first autumnal equinox the year of sowing (cut 2 weeks before equinox)
Char. 2: Plant: natural height 6 weeks after the first autumnal equinox the year of sowing (cut 3 weeks after equinox)
Char. 4: Plant: natural height in spring (1 month after beginning of growing the year after sowing)
Char. 14: Plant: natural height 2 weeks after second autumnal equinox the second year (cut 2 weeks before equinox)
Char. 15: Plant: natural height 6 weeks after second autumnal equinox the second year (cut 3 weeks after equinox)

It is recommended that some of the following varieties have the same notes to ensure that descriptions are consistent:

Maverick	Dormancy rating 1	1
Vernal	Dormancy rating 2	2
Boja, Ranger	Dormancy rating 3	3
Legend, Mercedes	Dormancy rating 4	4
Archer	Dormancy rating 5	5
Abi 700, Dorine	Dormancy rating 6	6
Sutter Oro	Dormancy rating 7	7
Maricopa, Carmen	Dormancy rating 8	8
CUF 101, Medina	Dormancy rating 9	9
UC-1887	Dormancy rating 10	10
UC-1465	Dormancy rating 11	11

Ad. 17: Resistance to *Verticillium albo-atrum*

(1) The seeds are pre-germinated by sowing them on wet blotting paper in Petri dishes.

(2) When the germs are 4 to 5 mm long, they should be transplanted to pots. (For example, 50 germs can be transplanted to a pot of 30 cm x 30 cm). It is recommended that 150 plants per variety be observed.

(3) The pots should be put in a greenhouse at 20°C for three months. During one month, the plants should be grown with a nutritive KNOP solution (250 ml per pot and twice per week).

KNOP solution for 20 liters:

(Nitrate de calcium)	$(NO_3)_2CaH_2O$	20g
(Nitrate de potassium)	NO_3K	5g
(Sulfate de magnesium)	$SO_4Mg_7H_2O$	5g
(Phosphate de potassium) mono potassique	PO_4H_2K	5g

(4) The plants are cut between 2 to 3 cm and are inoculated one month later.

(5) The inoculum should be obtained after three weeks of culture made on the following substrate:

Saccharose	20 g
Extract of crystallizable malt	5 g
Citric acid	25 mg
Malic acid	25 mg
Iron chelate	20 mg
SO_4Mn2H_2O	3 mg
SO_4Cu5H_2O	3 mg
H_3BO_3	4 mg
SO_4ZN7H_2O	3 mg
KNOP solution	made up to 1000 ml

After the inoculum has been ground with a mixer, the suspension should contain 10^5 spores by mm^3.

(6) Contamination is by clipping the plants down to between 4 and 5 cm with scissors that have previously been dipped into the suspension.

(7) The pots are immediately transferred to a chamber with a high relative humidity between 80 and 100%. The temperature should be 17°C and the light intensity between 10000 and 15000 lux.

(8) The observations should be made 45 days later. To each plant one of the following notes is attributed:

4	dried plant
3	one stunted stem on the plant
2	dried leaf
1	enlightened veins
0	absence of symptoms

For each variety, the mean is calculated from the total of the notes divided by the number of plants observed.

(9) It is recommended that the following varieties have always the same notes to ensure that the descriptions are consistent:

Medalfa		3
Europe, Derby	medium	5
Vertus	high	7

Ad. 18: Resistance to *Ditylenchus dipsaci*

(1) Seeds are abraded, disinfected (15 minutes in Metalaxyl 1g/L) and pregerminated by sowing them in vermiculite (2000 seeds are sown to have 300 seeds germinated). It is recommended that 150 plants per variety be observed.

(2) After 4.5 days at 19°C, 14 hours of photoperiod, the seedling (the length of the root is nearly 1 cm) should be laid on soaked blotting paper of 240g (2 strips of 40 x10 cm). The seedlings are deposited on the 1/3 median of the part superior of the strip, only the cotyledons must not be on the paper. The two extremities of the superior strip are folded on the roots. The second strip of blotting paper is beginning the roll up. For each variety 16 rolls of 20 seedlings are done. The rolls are deposited in pots of 30 x 30cm, with water (1 cm deep) one variety per pot.

(3) The pots should be put in a climatic chamber at 19°C, 12 hours of photoperiod and 80% of humidity.

(4) Two days after, when the cotyledons are well opened, the inoculation is done with a micro pipette. On each seedling, deposit a drop of 20 micro litres containing 50 nematodes between the two cotyledons and mix with carbomethylcellulose at 40%. 15 rollers per genotype are inoculated.

The humidity is set is at 100% for 4 days and reduced progressively to 80% over the 2 following days.

(5) Observations should be made between 14 and 21 days after the inoculation. To each plant one of the following expressions is attributed:

-puffed seedling (sensitive seedling)
-stopped growth seedling (resistant seedling)
-seedlings without symptoms
-dead seedling
-indeterminate seedling

For each variety, the percentage is calculated from the total of the number of puffed seedlings divided by the sum of puffed seedling + stopped growth seedling + seedlings without symptoms.

(6) It is recommended that the following varieties have always the same notes to ensure that the descriptions are consistent:

Europe	low	3
Vertus	high	7

Ad. 19 : Resistance to *Colletotrichum trifolii* Bain and Essary (Anthracnose)

(Based on standard test guidelines as published by the North American Alfalfa Improvement Conference)

Plant Culture:

Container	10 cm plastic pots or flats
Medium	Potting soil mix
Temp/Light	23°C; 16+ hour day length
No. of Plants	50 per replication
No. of Reps	4 minimum
Other	Control insects and fertilize as necessary

Inoculum Culture:

Source	Infected stem tissue
Storage	Soil or silica gel (7)
Temperature	4°C
Storage Life	Up to several years

Inoculation Procedure:

Age of Plant	7-14 days (take stand counts at 7 days)
Type of Inoc.	Spore suspension with 2 drops Tween per L. distilled water, taken from 7 days old cultures incubated at 23°C on half strength oatmeal agar.
Concentration	2 X 10-6 spores per ml
Method	Spray to runoff, approx. 3 ml or 5 to 10 ml per flat; place in mist chamber to maintain 100% R.H. for 48 hours 23°C
Incubation:	
Location	Growth room or green house at 23°C
Age at Rating	10 to 14 days after inoculation

State of Expression	Example varieties (Race 1)
Highly resistant (>50%)	Sequel HR
Resistant (31-50°)	Trifecta
Moderately resistant (15-30°)	
Low resistant (6-14°)	Venus
Susceptible (0-<6°)	Hunter River

Rating

Resistance is assessed as in percent seedlings surviving 10 to 14 days after inoculation.

Check varieties (Race 1)

	Approximate Expected Resistance (%)	Acceptable Range of Reaction (%)
Resistant		
Arc	65-70	45-80
Saranac AR	45	40-60
Sequel HR	50	30-65
Susceptible		
Saranac	1	0-5
Hunter River	10	0-15

Values for resistant standards are percent survivors.

Ad. 20: Resistance to *Phytophtora medicaginis* (Hansen and Maxwell) (Phytophtora root rot).

(Based on standard test guidelines as published by the North American Alfalfa Improvement Conference)

Plant Culture:

Container	Seedling cavities or flat within a water reservoir or a deep tub with a single drainage hole which is capable of being plugged
Media	Coarse vermiculite or a porous soil mix (eg. 3 :2 sphagnum-based soilless mix: perlite); provide a coarse drainage layer (eg. Gravel); pure sand medium is not desirable.
Temp/Light	20-24°C; 12-16 hr. day length
No. of Plants	50-70 per replication
No. of Reps.	3 minimum

Inoculum Culture:

Source	Seedlings grown on infested soil
Storage	Corn meal or V-8 juice agar.
Temperature	4-12°C
Storage life	6 months if hydrated

Inoculum Procedure:

Age of Plant	10-12 days (when first trifoliate begins expansion)
Type of Inoc.	Zoospore suspension or comminuted mycelium
Production	Produce zoospores as per Miller and Maxwell (1984); or 9-day-old V-8 agar cultures of mycelium may be chopped in a blender for 10 secs
Concentration	Aprox. 50 zoospores or 1 ml. Chopped mycelium per seedling; mycelium prepared as: 1 culture (9 cm diam.) in 1 l water
Method	For zoospores: Presaturate the soil mix and drench inoculum over the seedlings; for mycelium: drench inoculum into shallow trench and then saturate the soil with water.

Incubation:

Location	Moderate green house or growth chamber

Plant counts	Count at full emergence (7-8 days after seeding)
Culture	Maintain flooded conditions for 2 days; keep moist until rated
Age at Rating	Rate when nearly all plants of susceptible check variety are stunted and dying, i.e. for zoospores: 10-12 days after inoculation; for mycelium: 14 days after inoculation.

State of Expression	Example varieties
Highly resistant (>50%)	Aquarius
Resistant (31-50°)	
Moderately resistant (15-30°)	Trifecta
Low resistant (6-14°)	
Susceptible (0-<6°)	Hunterfield

Rating

Resistant – Vigorously growing plants with only slight to no necrosis of tap and secondary roots; hypocotyls area sound with slight to no chlorosis of cotyledons.

Susceptible – Stunted or dead plants with moderate to severe necrosis of roots, hypocotyls and cotyledons.

Check varieties

	Approximate Expected Resistance (%)	Acceptable Range of Reaction (%)
Highly Resistant		
WAPH-1	55	50-60
Aquarius	55	45-70
Resistant		
Agate	33	25-40
Susceptible		
Saranac	1	0-5
Hunterfield	4	0-7

Ad. 21 : Resistance to *Acyrthosiphon kondoi* Shinji (Blue Alfalfa Aphid)

(Based on standard test guidelines as published by the North American Alfalfa Improvement Conference)

Plant Culture:

Container	Flats (6 x 31 x 55 cm or similar size)
Medium	Soil mix (e.g. 8 parts sand; 3 peat; 3 pearlite; 1.4% by vol. lime)
Temp/Light	22 ± 4°C; 16+ hour day length
No. of Plants	50 to 70 per replicate in rows 3 cm apart
No. of Reps	3 minimum
Other	Scarify seed and treat with fungicide to prevent damping-off; sow seed 1cm deep and cover with vermiculite

Aphid Colony:

Source	Colony consisting of blend of several field collections from area of adaptation, replenished annually
Rearing	Susceptible alfalfa in greenhouse (eg. PA-1)
Temp/Light	22 ±4°C and 16+ hour day length

Infestation Procedure:

Age of Plant	1 day after emergence; cotyledon stage; count seedlings at time of infestation
Method	Sprinkle aphids onto seedlings
Rate	Minimum of 2 aphids per seedling
Length	Approx. 21 days; spray with insecticide to terminate infestation; rate plants 7 to 10 days after spraying
Other	It is critical to maintain temperature within the range of 18 to 26°C for optimal aphid reproduction and effective resistance evaluation

Correlation to Field Reaction:
Although there have not been extensive comparisons of greenhouse and field results, it appears that levels of resistance are comparable in both situations.

Biotypes:
Although biotypes of blue alfalfa aphid are not proven to exist, there is evidence of differential reactions to resistant plants in different locations.

State of Expression	Example varieties
Highly resistant (>50%)	Aurora
Resistant (31-50°)	
Moderately resistant (15-30°)	Siriver
Low resistant (6-14°)	
Susceptible (0-<6°)	Hunter River

Rating

1 Resistant	Tall, normal trifoliates
2 Resistant	Tall, small trifoliates
3 Resistant	Moderately tall, small, crinkled trifoliolates
4 Susceptible	Short; small, crinkledtrifoliolates, usually chlorotic
5 Susceptible	Dead (= total emerged – classes 1 to 4)

Check varieties

	Approximate Expected Resistance (%)	Acceptable Range of Reaction (%)
Resistant		
CUF-101	55	40-65
Aurora	60	45-75
Susceptible		
PA-1	10	0-5
Caliverde	3	0-5

Values for resistant standards are totals for rating 1 to 3. Percentage of plants surviving may be higher but may include many plants with little or no resistance.

Ad. 22: Resistance to *therioaphis maeulata* (Buckton) (Spotted Alfalfa Aphid).

(Based on standard test guidelines as published by the North American Alfalfa Improvement Conference)

Plant Culture:

Container	Flats (6 x 31 x 55 cm or similar size)
Medium	Soil mix (e.g. 8 parts sand; 3 peat; 3 pearlite; 1.4% by vol. lime)
Temp/Light	26 ± 4°C; 18+ hour day length
No. of Plants	50 to 70 per replicate in rows 3 cm apart
No. of Reps	3 minimum
Other	Scarify seed and treat with fungicide to prevent damping-off; sow seed 1cm deep and cover with vermiculite

Aphid Colony:

Source	Colony consisting of blend of several field collections from area of adaptation, replenished annually
Rearing	Susceptible alfalfa in greenhouse (eg. Arc, Caliverde)
Temp/Light	26± 4°C and 18 hour day length

Infestation Procedure:

Age of Plant	7 to 8 days after emergence; unifoliolate stage; count plants at time of infestation
Method	Sprinkle aphids onto plants
Rate	Minimum of 2 aphids per plant
Length	Approx. 18 days or when 85% of susceptible check plants are dead and resistant check is within the expected range; spray with insecticide to terminate infestation; rate plants 10 to 15 days after spraying

Correlation to Field Reaction:
Field performance of alfalfa selected for resistance to spotted alfalfa aphid has conformed closely with expected results based on greenhouse evaluations.

Biotypes:
Performance of resistant cultivars may vary depending upon the biotype(s) present. It would be advisable to test cultivars against aphid populations in areas where they will be grown.

State of Expression	Example varieties
Highly resistant (>50%)	Aurora
Resistant (31-50°)	
Moderately resistant (15-30°)	Trifecta
Low resistant (6-14°)	
Susceptible (0-<6°)	Hunter River

Rating

1-2 Resistant	Plant has formed at least one trifoliate
3 Susceptible	Plant has developed very little during infestation
4 Susceptible	Plant living but has formed no trifoliolates
5 Susceptible	Dead (= total emerged – classes 1 to 4)

Check varieties

	Approximate Expected Resistance (%)	Acceptable Range of Reaction (%)
Resistant		
CUF-101	60	45-75
Baker	50	35-65
Aurora	65	45-80
Susceptible		
Arc	3	0-5
Caliverde	3	0-5
Hunter River	3	0-5

Values for resistant standards are totals of 1st and 2nd. Percentage of plants surviving may be higher but may include many plants with little or no resistance.

9. Literature

Caubel G., Genier G., Bossis M. 1978. "Données utiles au sélectionneur pour améliorer la résistance des luzernes à l'égard des maladies et ravageurs ». Publication INRA.

GEVES-SNES. « Mode opératoire. Test de résistance de la Luzerne au *Verticillium albo-atrum* ». MO/ANA/PAT/TRS/405. Publication GEVES-SNES

GEVES-SNES. « Mode opératoire. Test de résistance des luzernes à *Ditylenchus dipsaci* ». MO/ANA/PAT/TRS/301. Publication GEVES SNES.

Gondran J. 1984. « La verticilliose de la luzerne : Détermination de l'agent causal, biologie du parasite répartition géographique, dégâts et méthode de lutte ». Thèse, université des sciences de Poitiers.

Leclercq D., Caubel G. 1991. « Résistance variétale de la luzerne au nématode des tiges *Ditylenchus dipsaci* (Kühn) Filipjev ; test d'évaluation et application en sélection ». Agronomie. 11, pages 603-612.

Montegano, B., Gensollen, V., and Lassalvy S. 2002. "Fall dormancy as a descriptor of Lucerne (*Medicago sativa* L.) varieties". 19th General Meeting of the European Grassland Federation. La Rochelle, France. Pages 452-453.

Roulier. G., Guy P. 1986. « Stades phénologiques de la luzerne, outil pour l'éleveur ». Le Sélectionneur Français. 37, pages 85-90.

Teuber, L.R.,Taggard, K.L., Gibbs, L.K., Mccaslin, M.H., Peterson, M.A., Barnes, D.K. 1998. « Fall Dormancy. In Standard tests to characterize alfalfa cultivars. 3rd ed. (amended 1998). North American Alfalfa Improvement Conference, Beltsville, MD. (Available on line at http://www.naaic.org/stdtests/Dormancy2.html) (Verified July 11, 2003).

10. Technical Questionnaire

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

	Application date: (not to be filled in by the applicant)

TECHNICAL QUESTIONNAIRE
to be completed in connection with an application for plant breeders' rights

1.1.1 *Latin Name*	*Medicago Sativa* L.	
1.1.2 Common Name	Lucerne	[ ]
1.2.1 *Latin Name*	*Medicago* x *varia* Martyn	
1.2.2 Common Name		[ ]

2. Applicant

Name	
Address	
Telephone No.	
Fax No.	
E-mail address	

Breeder (if different from applicant)

3. Proposed denomination and breeder's reference

Proposed denomination (if available)	
Breeder's reference	

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

[#]4. Information on the breeding scheme and propagation of the variety

4.1 Breeding scheme

Variety resulting from:

4.1.1 Crossing

(a) controlled cross [ ]
(please state parent varieties)
(b) partially known cross [ ]
(please state known parent variety(ies))
(c) totally unknown cross [ ]

4.1.2 Mutation [ ]
(please state parent variety)

4.1.3 Discovery [ ]
(please state where, when and how developed)

4.1.4 Other [ ]
(please provide details)

4.2 Method of propagating the variety

[#] Authorities may allow certain of this information to be provided in a confidential section of the Technical Questionnaire.

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

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

	Characteristics	Example Varieties	Note
5.1 (6)	**Flower: frequency of plants with very dark blue violet flowers**		
	absent or very low	Diane	1
	low	Sanditi	3
	medium	Andela	5
	high	Orca	7
	very high		9
5.2 (7)	**Flower: frequency of plants with variegated flowers**		
	absent or very low	Symphonie	1
	low	Luzelle, Letizia	3
	medium	Franken Neu, Likarlu	5
	high		7
	very high		9
5.3 (8)	**Flower: frequency of plants with cream, white or yellow flowers**		
	absent or very low	Europe	1
	low		3
	medium	Likarlu	5
	high		7
	very high		9

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

	Characteristics	Example Varieties	Note
5.4 (16)	**Plant: tendency to grow during winter**		
	very weak		1
	very weak to weak	Vernal	2
	weak	Boja, Ranger	3
	weak to medium	Legend, Mercedes	4
	medium	Archer, Europe	5
	medium to strong	Abi 700, Dorine	6
	strong	Sutter Oro	7
	strong to very strong	Maricopa, Carmen	8
	very strong	CUF 101, Medina	9

6. Similar varieties and differences from these varieties

Please use the following table and box for comments to provide information on how your candidate variety differs from the variety (or varieties) which, to the best of your knowledge, is (or are) most similar. This information may help the examination authority to conduct its examination of distinctness in a more efficient way.

Denomination(s) of variety(ies) similar to your candidate variety	Characteristic(s) in which your candidate variety differs from the similar variety(ies)	Describe the expression of the characteristic(s) for the **similar** variety(ies)	Describe the expression of the characteristic(s) for **your** candidate variety
Example		*(example to be inserted)*	*(example to be inserted)*

Comments:

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

[#]7. Additional information which may help in the examination of the variety

7.1 In addition to the information provided in sections 5 and 6, are there any additional characteristics which may help to distinguish the variety?

Yes [ ] No [ ]

(If yes, please provide details)

7.2 Special conditions for the examination of the variety

7.2.1 Are there any special conditions for growing the variety or conducting the examination?

Yes [ ] No [ ]

7.2.2 If yes, please give details:

7.3 Other information

8. Authorization for release

(a) Does the variety require prior authorization for release under legislation concerning the protection of the environment, human and animal health?

Yes [ ] No [ ]

(b) Has such authorization been obtained?

Yes [ ] No [ ]

If the answer to (b) is yes, please attach a copy of the authorization.

[#] Authorities may allow certain of this information to be provided in a confidential section of the Technical Questionnaire.

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:

9. Information on plant material to be examined.

9.1 The expression of a characteristic or several characteristics of a variety may be affected by factors, such as pests and disease, chemical treatment (e.g. growth retardants or pesticides), effects of tissue culture, different rootstocks, scions taken from different growth phases of a tree, etc.

9.2 The plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If the plant material has undergone such treatment, full details of the treatment must be given. In this respect, please indicate below, to the best of your knowledge, if the plant material to be examined has been subjected to:

(a) Microorganisms (e.g. virus, bacteria, phytoplasma) Yes [ ] No [ ]

(b) Chemical treatment (e.g. growth retardant or pesticide) Yes [ ] No [ ]

(c) Tissue culture Yes [ ] No [ ]

(d) Other factors Yes [ ] No [ ]

Please provide details of where you have indicated “yes”.

……

10. I hereby declare that, to the best of my knowledge, the information provided in this form is correct:

Applicant’s name

Signature Date

[End of document]