

**Technical Working Party for Agricultural Crops****TWA/46/5****Forty-Sixth Session  
Hanover, Germany, June 19 to 23, 2017****Original:** English  
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**IMPACT OF ENDOPHYTES ON DUS CHARACTERISTICS IN GRASSES***Document prepared by the Office of the Union**Disclaimer: this document does not represent UPOV policies or guidance***EXECUTIVE SUMMARY**

1. The purpose of this document is to report on developments concerning the impact of endophytes on DUS characteristics in grasses.
2. The TWA is invited to consider the information to be presented at its forty-sixth session on the impact of endophytes on DUS characteristics in grasses, as presented in Annexes I and II to this document.

**BACKGROUND**

3. The TWA, at its forty-fifth session, held in Mexico City, from July 11 to 15, 2016, received a presentation on the “Impact Analysis of Endophytes on the Phenotype of Varieties of *Lolium perenne* and *Festuca arundinacea*” by an expert from the Community Plant Variety Office of the European Union (CPVO), a copy of which is provided in the Annex to document TWA/45/24 (see document TWA/45/25 “Report”, paragraphs 67 to 70).
4. The TWA noted there had been no interaction between the endophytes studied and expression of the DUS characteristics on the crops studied. The TWA agreed that it would not be possible to make a general recommendation on the effect of endophytes in DUS characteristics due to the possibility of positive interaction between other endophytes and the expression of DUS characteristics.
5. The TWA noted the report that New Zealand would consider the requirement for endophyte-free plant material for DUS examination and welcomed the offer to make a presentation on the outcome of discussions to the TWA at its session in 2017.
6. The TWA welcomed the offer by the European Union to make a presentation on the outcome of discussions in the CPVO and the offer by Mexico to make a presentation on the impact of endophytes on DUS characteristics in grasses at its forty-sixth session.
7. The Technical Committee (TC), at its fifty-third session, held in Geneva from April 3 to 7, 2017, noted developments in the TWA concerning the impact of endophytes on DUS characteristics in grasses (see document TC/53/31 “Report”, paragraph 106).

INFORMATION TO BE PRESENTED AT THE FORTY-SIXTH SESSION OF THE TWA

8. The Annexes to this document contain the following:

ANNEX I “Impact of endophyte on DUS characteristics of grasses: New Zealand’s experience of fungal endophyte in grass variety DUS testing”, document prepared by an expert from New Zealand;

ANNEX II “Impact of endophytes on DUS characteristics in grasses”, presentation prepared by an expert from the European Union.

*9. The TWA is invited to consider the information to be presented at its forty-sixth session on the impact of endophytes on DUS characteristics in grasses, as presented in Annexes I and II to this document.*

[Annexes follow]

IMPACT OF ENDOPHYTE ON DUS CHARACTERISTICS OF GRASSES:  
NEW ZEALAND'S EXPERIENCE OF FUNGAL ENDOPHYTE IN GRASS VARIETY DUS TESTING

Document prepared by an expert from New Zealand

Since 2011 New Zealand has required that seed submitted for DUS testing has less than 5% of seed infected with fungal endophyte. This policy was implemented so as to eliminate potential sources of variation between varieties, and to address concerns from some breeders and researchers that endophytes may impact grass variety morphology. In the absence of clear evidence whether endophyte presence affects plant morphology, it was decided this potential source of variation was best dealt with by removing endophyte from all varieties tested.

In practice this approach has caused difficulties. Frequently in New Zealand grass varieties (particularly those of *Lolium* species) are produced containing a matched endophyte. Seed without endophyte may not be readily available leading to the need to remove endophyte from the seed sample by heat treatment. Heat treatment decreases the long term viability of seed so that seed as young as four years old can have significantly reduced germination rates. It is not desirable that the official seed sample that forms part of the identity of the variety has decreased longevity.

Older unprotected varieties which are not marketed with a matched endophyte may still have "wild" endophyte present in the seed, which the variety maintainer may or may not be aware of.

The 2013-2015 Community Plant Variety Office of the European Union (CPVO) research on the impact of endophytes on the phenotype of *L. perenne* and *F. arundinacea* conducted in Northern Ireland, Germany, and France provided evidence on the lack of effect of endophyte on DUS characteristics. The study found no evidence that the absence or presence of endophyte significantly effects morphological identity. Greater differences were found between different seed samples of the same variety, than between the same seed sample with and without endophyte. Care needs to be taken in extrapolating the results beyond the grass and endophyte varieties used, the particular DUS characteristics examined, and the trial locations used.

The New Zealand Plant Variety Rights Office discussed the results of the CPVO research with the grass breeding industry in New Zealand as part of assessing whether it remained appropriate to continue the practice of requiring grass seed submitted for DUS testing to be endophyte free. A number of benefits and risks were identified for removing the requirement for endophyte free seed:

#### Benefits

- Avoid extra work for applicants in providing an endophyte free seed sample.
- Avoid reduced seed viability of the official seed sample over time due to heat treatment to remove endophyte.
- Potentially fewer treatments for pest control are required during growing trials.

#### Risks

- Despite evidence and experience so far, some endophytes strains may significantly influence DUS characteristics. In particular new endophytes varieties developed in the future may influence morphology.
- That any new characteristics added to DUS testing in the future may be influenced by endophytes.
- That New Zealand test reports would no longer be acceptable to other UPOV members.

There was unanimous support from grass breeders we spoke with to remove the requirement for endophyte free seed. This was a significant change in their views since 2011.

New Policy

It was decided that the advantages of removing the requirement for endophyte free seed outweighed the disadvantages. In January 2017 the New Zealand Plant Variety Rights Office altered its policy so that it is now acceptable to supply seed with viable endophyte present. Because of concerns that some endophyte grass variety combinations may in future be determined to affect some DUS characteristics there remains a requirement for applicants to supply information on the endophyte status of their grass seed. As part of completing the technical questionnaire for ryegrass varieties applicants must indicate whether viable endophyte is present, the variety of any such endophyte, and the approximate level (percentage) of infected seed with viable endophyte.

[Annex II follows]

IMPACT OF ENDOPHYTES ON DUS CHARACTERISTICS IN GRASSES

Presentation prepared by an expert from the European Union



TWA/46 Session

**Impact of Endophytes on  
DUS characteristics in grasses**

Hannover, June 2017

R&D project 2013 - 2015

**Impact analysis of Endophytes on the Phenotype  
of varieties of *L. perenne* and *F. arundinacea***

Report Team (DUS observations and analysis):

- Trevor J. Gilliland (AFBI, GB, co-ordinator), Lp
- Susanne Wöster (Bundessortenamt, DE), Lp
- Frédéric Lafaille (GEVES, FR), Fa

European Seed Association (Breeders)

- Niels Roulund (DLF Trifolium) + Stéphane Charrier (Barenbrug)

CPVO coordinator: Anne Weitz

Funding: CPVO and ESA

## Introduction

### Plant material requirements for DUS test:

(*Species Lolium perenne and Festuca arundinacea*)

- Does the Endophyte have an impact on the phenotype of the infested plant and thus on the DUS test?
- Can it sufficiently modify the morphology of a variety to make it distinct from a sample containing no endophyte (circumventing protection)?
- Must seeds for DUS be free of endophyte?



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## Project design

### Locations:

Perennial ryegrass (all diploid, amenity):

- AFBI, GB
- Bundessortenamt, Germany

Tall fescue (probably hexaploid):

- GEVES, France

### Endophytes: examined accessions

E+ = 100% Endophyte inoculated

E - = Endophyte free (0%)

DEF = Standard sample of the protected variety



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## Project design

**Duration:** 2 growing cycles (2013/2014 + 2014/2015)

**Endophytes: examined accessions**

E+ = 100% Endophyte inoculated

E - = Endophyte free (0%)

DEF = Standard sample of the protected variety

**Nr. of plants:** 60 per variety per growing status E –  
60 per variety per growing status E +  
(a different 60 plants used for each growing cycle)

**Varieties** (coded)

Lp: Binnian, Donard, Croob, Gullion

Fa: Anorra, Divis, Trostan, Meelbeg



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## Results

**Differences tested at significance levels of 1% and 5%**

### Results

- 132 pairwise comparisons

### Implications of E + versus E –

- **No evidence that absence/presence of endophyte changes morphological identity significantly**



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## Conclusions

### Options discussed with CPVO crop experts

### Requirements for plant material used for DUS test

- a.) Retain an endophyte free seed requirement
- b.) Accept seeds with endophyte infection
- c.) Require no information on endophyte presence/absence



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## Conclusions

### Options b.) retained for rye grass and tall fescue

☞ **Accept seeds with endophyte infection for DUS test**

#### TQ modified:

TQ 9.5 In case the variety is infected with endophytes, please indicate for information purpose the level of infection

- Category I : < 10% endophytes
- Category II : 10 < % < 85 endophytes
- Category III : > 85% endophytes
- not tested**

Watch out: requirements for VCU test might differ!



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**THANK YOU  
FOR YOUR ATTENTION**



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[End of Annex II and of document]