# 2016 AOSA Rules Change Proposal 17

**PURPOSE OF PROPOSAL**

To replace the current AOSA formula to determine the proportions of annual and perennial ryegrass in the pure (ryegrass) seed component from the purity analysis based on the fluorescence test [AOSA Rules, Vol. 1. sec. 5.2.b (2) **Fluorescence test of ryegrass seedlings].**

**PRESENT RULE**

**5.2.b. Fluorescence tests**

(2) **Fluorescence test of ryegrass seedlings:** To determine the percentage of ryegrass seedlings with fluorescent roots.

A fluorescence test shall be made on all samples of ryegrass for which the percentage of perennial ryegrass (*Lolium perenne*) and/or annual (Italian) ryegrass (*L. multiflorum*) is to be reported. The seedlings shall be grown on filter paper and the number of fluorescent seedlings determined under ultraviolet light at the end of the germination period. Fluorescence results are to be reported as test fluorescence (TFl) to two decimal places as follows:

$$\frac{Number\ of\ normal\ fluorescent\ seedlings}{Total\ number\ of\ normal\ seedlings}\ x\ 100 = \%\ TFl$$

**Application of fluorescence test results to sample purity:** the following formula shall be used to determine the proportions of annual and perennial ryegrass in the pure (ryegrass) seed component from the purity test. For tolerances, see section 14.4, Table 14H.

The percentage of perennial ryegrass is calculated as follows:

$$\%\ Perennial\ Ryegrass = \frac{\%\ VFL\ (annual)\ -\ \%\ TFL}{\%\ VFL\ (annual)\ -\ \%\ VFL\ (perennial)}\ x\ \%\ pure\ ryegrass$$

Where VFl is varietal fluorescence and TFl is test fluorescence.

% annual ryegrass = % pure ryegrass – % perennial ryegrass

When testing a variety of perennial ryegrass, if the test fluorescence (TFl) is equal to or less than the level described for the variety [VFl (perennial)], do not apply the formula. In this case, all pure ryegrass shall be considered to be perennial.

When testing a variety of annual ryegrass, if the test fluorescence (TFl) is equal to or greater than the level described for the variety [VFl (annual)], do not apply the formula. In this case, all pure ryegrass shall be considered to be annual.

A list of fluorescence level descriptions (VFl) for perennial ryegrass varieties that are more than zero percent fluorescent and annual ryegrass varieties that are less than 100 percent fluorescent is maintained and published by the National Grass Variety Review Board of the Association of Official Seed Certifying Agencies (AOSCA).

If the variety being tested is not stated or the fluorescence level has not been described, the fluorescence level shall be considered to be zero percent for perennial ryegrass and 100 percent for annual ryegrass.

Both VFl (annual) and VFl (perennial) values must always be entered in the formula. If a perennial ryegrass variety is being tested, the VFl (annual) value is 100 percent. If an annual ryegrass variety is being tested, the VFl (perennial) value is 0 percent. For blends, the fluorescence level shall be interpolated according to the portion of each variety claimed to be present.

***Example:***

Interpolation for ryegrass variety blends in which the varieties have different described levels of fluorescence (VFI).
Label: Variety A perennial ryegrass = 58.63%
Variety B perennial ryegrass = 39.42%
Total perennial ryegrass labeled = 98.05%

Variety A is not included in the description list and so is not considered to be 0% fluorescent.
Variety B is described as 2.50% fluorescent.

Fluorescence level for perennial ryegrass to be inserted in the formula %VFI (perennial)

$$(58.63\% \div 98.05 \times 0\%) + (39.42\% \div 98.05\% \times 2.5\%) = 1.01$$

**Examples:**

Application of fluorescence test results to sample purity:

**Example 1**

Perennial ryegrass

*Varity not stated in label*

Perennial variety fluorescence considered to be 0%
Test Fluorescence=1.88%
Pure Ryegrass=98.56%

$$\%\text{Perennial ryegrass} = \frac{100\% - 1.88\%}{100\% - 0\%} \times 98.56\% =$$

$$\frac{98.12\%}{100\%} \times 98.56\% = 0.9812 \times 98.56\% = 96.71\%$$

%Annual ryegrass=98.56%-96.71%=1.85%

**Example 2**

Perennial ryegrass

*With fluorescence level on the AOSCA list*
Variety Fluorescence (Perennial) =1.50%

Test Fluorescence=1.88%
Pure Ryegrass=98.56%

$$\%\text{Perennial ryegrass} = \frac{100\% - 1.88\%}{100\% - 1.5\%} \times 98.56\% =$$

$$\frac{98.12\%}{98.50\%} \times 98.56\% = 0.9961 \times 98.56\% = 98.18\%$$

%Annual ryegrass=98.56%-98.18%=0.38%

**Example 3**

Annual ryegrass
*With fluorescence level on the AOSCA list*
Variety Fluorescence (annual) =100%
Test Fluorescence=98.06%
Pure Ryegrass=99.23%

$$\%\text{Perennial ryegrass} = \frac{100\% - 96.06\%}{100\% - 0\%} \times 99.23\%$$

$$\frac{3.94\%}{100\%} \times 99.23\% = 0.0394 \times 99.23\% = 3.91\%$$

%Annual ryegrass=99.23%-3.91% =95.32%

**Example 4**

Annual ryegrass
*With fluorescence level on the AOSCA list*
Variety Fluorescence (annual) =90%
Test Fluorescence=84.72%
Pure Ryegrass=98.41%

$$\%\text{Perennial ryegrass} = \frac{90\% - 84.72\%}{90\% - 0\%} \times 98.41\%$$

$$\frac{5.28\%}{90\%} \times 98.41\% = 0.0587 \times 98.41\% = 5.77\%$$

%Annual ryegrass=98.41%-5.77% = 92.64%

**Example 5**

Mixture of annual and perennial ryegrass
*Each with fluorescence level descriptions on the AOSCA list*
Variety fluorescence (annual) = 90%
Variety fluorescence (perennial) = 4%

Test fluorescence = 81.43%
Pure ryegrass = 98.51%

$$\%\text{Perennial ryegrass} = \frac{90\% - 81.43\%}{90\% - 4\%} \text{ x } 98.51\%$$

$$\frac{8.57\%}{86\%} \text{ x } 98.51\% = 0.0997 \text{ x } 98.51\% = 9.82\%$$

% Annual ryegrass = 98.51-9.52% = 88.69%

**Example 6**

Grass mixture: annual ryegrass, variety not stated,
And two varieties of perennial ryegrass, *each with fluorescence level descriptions the AOSCA list*

| | | | |
|---|---|---|---|
| Label: | Annual ryegrass | | 58.41% |
| | Creeping red fescue | 18.41% | |
| | Variety A Perennial ryegrass | 9.78% | |
| | Variety B Perennial ryegrass | 9.56% | |
| Laboratory Test Results: | | | |
| | Test fluorescence = | 81.91% | |
| | Pure ryegrass = | 78.06% | |
| | Annual ryegrass, variety not stated = | Considered to be 100% | |
| AOSCA List: | Variety A perennial ryegrass VFI = | 0% | |
| AOSCA List: | Variety B perennial ryegrass VFI = | 2.50% | |

For the use in the equation, VFI (perennial) must be interpolated:

$$\% \text{ VFI (perennial)} = \left(\frac{9.78\%}{9.78\% + 9.56\%} \text{ x } 0\%\right) + \left(\frac{9.56\%}{9.78 + 9.56\%} \text{ x } 2.50\%\right) =$$

$$\left(\frac{9.78\%}{19.34\%} \text{ x } 0\%\right) + \left(\frac{9.56\%}{19.34\%} \text{ x } 2.50\%\right) = (0.5057 \text{ x } 0\%) + (0.4943 \text{ x } 2.50\% = 1.24\%$$

$$\% \text{ Perennial ryegrass} = \frac{100\% - 81.91\%}{100\% - 1.24\%} \text{ x } 78.061\%$$

$$\frac{18.09\%}{98.76\%.} \text{ x } 78.06\% = 0.1832 \text{ x } 78.06\% = 14.30\%$$

% Annual ryegrass = 78.06% - 14.30% = 63.76%

**PROPOSED RULE**

**5.2.b. Fluorescence tests**

(2) **Fluorescence test of ryegrass seedlings:** To determine the percentage of ryegrass seedlings with fluorescent roots.

A fluorescence test shall be made on all samples of ryegrass for which the percentage of perennial ryegrass (*Lolium perenne*), annual and Italian ryegrass (*L. multiflorum*) is to be reported. The seedlings shall be grown on filter paper and the number of fluorescent seedlings determined under ultraviolet light at the end of the germination period[1]. Fluorescence results are to be reported as test fluorescence (TFL) to two decimal places as follows:

$$\frac{Number\ of\ normal\ fluorescent\ seedlings}{Total\ number\ of\ normal\ seedlings}\ x\ 100 = \%\ TFl$$

**Application of fluorescence test results to sample purity:** the following formula shall be used to determine the proportions of annual and perennial ryegrass in the pure (ryegrass) seed component from the purity analysis. For tolerances, see section 14.4, Table 14H.

**In the case of perennial ryegrass samples:**

The percentage of annual ryegrass present in a perennial ryegrass sample is calculated as follows:

% Annual ryegrass = (%TFL - %$VFL_P$)/100% x % pure ryegrass

Where $VFL_P$ is varietal fluorescence (for the perennial variety) and TFL is test fluorescence.

% Perennial ryegrass = % pure ryegrass – % annual ryegrass

**In the case of annual ryegrass samples:**

The percentage of perennial ryegrass in an annual ryegrass sample is calculated as follows:

% Perennial ryegrass = (%$VFL_A$ - %TFL)/100% x % pure ryegrass

Where $VFL_A$ is varietal fluorescence in percentages (for the annual variety) and TFL is test fluorescence in percentages.

% Annual ryegrass = % pure ryegrass – % perennial ryegrass

**Important considerations:**

When testing a variety of perennial ryegrass, if the test fluorescence (TFL) is ***equal to or less*** than the described $VFL_P$ of the variety, do not apply the formula. In this case, all pure ryegrass shall be considered perennial type.

When testing a variety of annual ryegrass, if the test fluorescence (TFL) is ***equal to or greater*** than the described $VFL_A$ of the variety, do not apply the formula. In this case, all pure ryegrass shall be considered annual type.

A list of variety fluorescence level (VFL) description for perennial and annual ryegrass varieties is maintained and published by the National Grass Variety Review Board (NGVRB) of the Association of

Official Seed Certifying Agencies (AOSCA).

If the variety is not stated in the NGVRB list, or if the VFL of a variety has not been described or stated in a label, the VFL shall be considered "0%" for perennial ryegrass and "100%" for annual ryegrass.

For blends, the fluorescence level shall be interpolated according to the portion of each variety claimed to be present. Based on label information, use the formula below to determine the VFL for a blend:

$$\%VFL_{(blend)} = \frac{\%\ var\ A}{\%\ total\ ryegrass} \times \%VFL_{(var\ A)} + \frac{\%\ var\ B}{\%\ total\ ryegrass} \times \%VFL_{(var\ B)} + \ldots\ldots$$

**Example 1. Perennial ryegrass variety with low VFL description**

The perennial ryegrass variety tested has a VFL description of 5.19%
The TFL result was found to be 9.44%
The pure ryegrass found in purity analysis was 99.79%
What are the percentages of annual and perennial ryegrass in the sample?

*For perennial ryegrass, the following formula must be used:*

% Annual ryegrass = (%TFL - $\%VFL_P$)/100% × % pure ryegrass

% Annual ryegrass = (9.44% – 5.19%)/100% × 99.79% = 0.0425 × 99.79% = 4.24%

% Perennial ryegrass = % pure ryegrass – % annual ryegrass

% Perennial ryegrass = 99.79% – 4.24% = 95.55%

**Example 2. Perennial ryegrass variety with high VFL description**

The perennial ryegrass variety tested has a VFL description of 81.78%
The TFL was found to be 91.64%
The pure ryegrass found in purity analysis was 99.65%

What are the percentages of annual and perennial ryegrass in this sample?

*For perennial ryegrass, the following formula must be used:*

% Annual ryegrass = (%TFL - $\%VFL_P$)/100% × % Pure ryegrass

% Annual ryegrass = (91.64% – 81.78%)/100% × 99.65% = 0.0986 × 99.65% = 9.83%

% Perennial ryegrass = % pure ryegrass – % annual ryegrass

% Perennial ryegrass = 99.65% – 9.83% = 89.82%

**Example 3. Perennial ryegrass variety with no VFL description**

The perennial ryegrass variety tested has no VFL description; therefore, VFL assumed to be 0%
The TFL was found to be 4.48%
The pure ryegrass found in purity analysis was 99.69%
What are the percentages of annual and perennial ryegrass in this sample?

*For perennial ryegrass, the following formula must be used:*

% Annual ryegrass = (%TFL - $\%VFL_P$) /100% × % pure ryegrass

% Annual ryegrass = (4.48% – 0.00%)/100% × 99.69% = 0.0448 × 99.69% = 4.47%

% Perennial ryegrass = % pure ryegrass – % annual ryegrass

% Perennial ryegrass = 99.69% – 4.47% = 95.22%

**Example 4. Annual ryegrass variety with VFL description**

The annual ryegrass variety tested has a VFL description of 99.05%
The TFL was found to be 95.83%
The pure ryegrass found in purity analysis was 99.37%
What are the percentages of perennial and annual ryegrass in this sample?

*For annual ryegrass varieties, the following formula must be used*:

% Perennial ryegrass = ($\%VFL_A$ - %TFL)/100% × % pure ryegrass

% Perennial ryegrass = (99.05% – 95.83%)/100% × 99.37% = 0.0322 × 99.37% = 3.20%

% Annual ryegrass = % pure ryegrass – % perennial ryegrass

% Annual ryegrass = 99.37% – 3.20% = 96.17%

**Example 5. Annual variety with no VFL description**

The annual ryegrass variety has no VFL description; therefore, VFL assumed to be 100%
The TFL was found to be 95.83%
The pure ryegrass found in purity analysis was 99.37%
What are the percentages of perennial and annual ryegrass in this sample?

*For annual ryegrass varieties, the following formula must be used*:

% Perennial ryegrass = ($\%VFL_A$ - %TFL)/100% × % pure ryegrass

% Perennial ryegrass = (100.00% – 95.83%)/100% × 99.37% = 0.0417 × 99.37% = 4.14%

% Annual ryegrass = % pure ryegrass – % Perennial ryegrass

% Annual ryegrass = 99.37% – 4.14% = 95.23%

**Examples with Blends and Mixtures**

**Clarifications for the following examples**

Blends and mixtures are prepared based on percentage by weight. However, the fluorescence values of VFL and TFL are determined based on percentages by number. Thus, extrapolating the "expected" VFL values of various species (i.e., annual and perennial) or varieties (within the same species) may not predict the "actual" VFL of the seeds in the bag correctly. Sources of variation between the "expected" and the "actual" VFL are: seed sizes of different species, germination values, variability of VFL within each variety, and sampling variation. These factors contribute to the variability between the expected, interpolated VFL value of a mixture and the actual, "observed", TFL value of the same mixture. Thus, the most reliable information on the actual fluorescent seeds in a mixture or a blend sample would be the TFL result of that sample. This is because the TFL integrates all variables and represents the fluorescence level of the blend or the mixture as it is in the bag, not based on the expected "ideal" VFL values description of each variety, or in some cases, based on random estimation of "0% for perennial varieties and "100%" of annual varieties.

When blending varieties within the same species with similar seed sizes and VFL values, using the extrapolated VFL may present a good approximation to the actual VFL of the blend in the bag (see example 6). However, when mixing annual (big seeds with high VFL) with perennial (small seeds with low VFL) ryegrass, extrapolating an "expected" VFL of the mixture based on percentage by weight would be misleading because of the large difference in seed sizes and VFL values which is calculated based on percentage by number. In such cases, the best predictor of the fluorescence level of the seeds inside the bag would be the TFL of the mixed product (see example 7, 8). If this is in doubt, simply conduct a supplementary test for more precise determinations.

**Example 6. Blend of perennial ryegrass varieties, with similar VFL descriptions**

*Label information:*
Perennial ryegrass Variety A = 29.53% pure seed
Perennial ryegrass Variety B = 24.39% pure seed
Perennial ryegrass Variety C = 22.75% pure seed
Perennial ryegrass Variety D = 21.08% pure seed

Total perennial ryegrass = 97.75%

Variety A perennial ryegrass tested has a VFL description of 0.81%
Variety B perennial ryegrass tested has a VFL description of 1.49%
Variety C perennial ryegrass tested has a VFL description of 0.65%
Variety D perennial ryegrass tested has a VFL description of 3.66%

The TFL was found to be 10.50%
The pure ryegrass found in purity analysis was 98.23%

What are the percentages of perennial and annual ryegrass in this sample?

The formula below must be used to determine the VFL for a blend:

$$\%VFL_{(blend)} = \frac{\%\ var\ A}{\%\ total\ ryegrass} \times \%VFL_{(var\ A)} + \frac{\%\ var\ B}{\%\ total\ ryegrass} \times \%VFL_{(var\ B)} + \ldots\ldots$$

*Calculation of VFL for the perennial blend*:

$$\%VFL_{p(blend)} = \frac{29.53}{97.75} \times 0.81_{(var\ A)} + \frac{24.39}{97.75} \times 1.49_{(var\ B)} + \frac{22.75}{97.75} \times 0.65_{(var\ C)} + \frac{21.08}{97.75} \times 3.66_{(var\ D)}$$

$\%VFL_{p(blend)} = 0.245 + 0.372 + 0.151 + 0.789 = 1.557$; $VFL_P = 1.56\%$

*Since this is a blend of all perennials (97.75%), the calculated VFL is 1.56%, and the TFL is 10.50%, this blend is behaving as a perennial, thus the perennial ryegrass formula can be used:*

For perennial varieties, the following formula must be used:

% Annual ryegrass = $(\%TFL - \%VFL_{P(blend)})/100\% \times$ % Pure ryegrass

% Annual ryegrass = $(10.50\% - 1.56\%)/100\% \times 98.23\% = 0.0894 \times 98.23\% = 8.78\%$

% Perennial ryegrass = % pure ryegrass – % annual ryegrass

% Perennial ryegrass = 98.23% – 8.78% = 89.45%

**Example 7. Mixture of unknown amount of annual and perennial ryegrass, both with VFL description**

The annual ryegrass variety has a VFL description of = 90%
The perennial ryegrass variety has a VFL description of 4.0%
The TFL found in the mixture is 81.43%
The pure ryegrass found in purity analysis was 98.51%

What are the percentages of perennial and annual ryegrass in this sample?

*The two varieties are mixed in unknown quantities. The 81.43% TFL (observed fluorescent seedlings) indicates that the majority of the ryegrass in the mixture appears to be annual type. Therefore, the formula of annual ryegrass must be applied.*

% Perennial ryegrass = $(\%VFL_A - \%TFL)/100\% \times$ % pure ryegrass

% Perennial ryegrass = $(90.00\% - 81.43\%)/100\% \times 98.51\% = 0.0857 \times 98.51\% = 8.44\%$

% Annual ryegrass = 98.51% – 8.44% = 90.07%

**Example 8. Mixture of annual and perennial ryegrass in the following proportions**

*Label List:*

| | |
|---|---|
| Annual ryegrass | 58.41% |
| Creeping red fescue | 18.41% |
| Variety A Perennial ryegrass | 9.78% |
| Variety B Perennial ryegrass | 9.56% |

The pure ryegrass component in the sample = 78.06%

The two perennial varieties have the following descriptions:
VFL of Variety A = 0%
VFL of Variety B = 2.50%
VFL of the annual ryegrass (variety not stated), therefore it should be considered 100%

The laboratory TFL results = 81.91%

What are the percentages of perennial and annual ryegrass in this sample?

*In this case, extrapolating the VFL for the mixture would be misleading. The 81.91% TFL of the mixture is the most reliable information and is closer to the VFL of the annual component (100%), indicating that the mixture is behaving as mostly annual ryegrass. Therefore, the formula of annual ryegrass can be applied.*

% Perennial ryegrass = $(\%VFL_A - \%TFL)/100\% \times$ % pure ryegrass

% Perennial ryegrass = $(100.00\% - 81.91\%)/100\% \times 78.06\% = 0.1809 \times 78.06\% = 14.12\%$

% Annual ryegrass = 78.06% – 14.12% = 63.94%

## HARMONIZATION STATEMENT

The Federal Seed Act (FSA) regulations use the current AOSA formula in section 5.2.b. The ISTA Rules and the CFIA Methods and Procedures for Seed Testing do not have any formulae.

**SUPPORTING EVIDENCE** -- *See also Appendix 17*

New perennial ryegrass (PRG) varieties with VFL values of 10, 20, 30, 50, and even 80% have been developed and are being marketed. This made us realize that the current AOSA formula does not work on such varieties and needs to be corrected.

The current AOSA formula works well when the PRG variety has a VFL value of "0" or around "0". However, as the VFL values of perennial varieties increase, the current formula overestimates the annual ryegrass and, conversely, underestimates the perennial ryegrass in the sample. The distorted results create problems to both seed produces and buyers and affect the "truth in labeling" principle.

The magnitude of distortion varies with the VFL level and the TFL results of the sample. For examples, in a PRG variety with 0% VFL, similar results are expected whether the current AOSA or the proposed formula is used. However, in a PRG variety with VFL value of 5.88%, the current AOSA formula overestimates annual type by 0.11 to 1.04% depending on TFL levels. In a PRG variety with VFL of 10.03%, the current AOSA formula overestimates the annual type by 0.20% to 1.05%. In a PRG variety with 48.69% VFL value, the current AOSA formula overestimates the annual type by 3.86% to 34.41%; and in a PRG variety with 81.78% VFL, the current AOSA formula overestimates the annual type by 11.62% to 63.27%. Conversely, the perennial type is underestimated by the same values.

On the other hand, when the proposed formula is used, the results are considered correct because only the "TFL-VFL" is considered as annual type, i.e., the excess fluorescent seedlings than those described in the VFL. In ARG samples, only those TFL values below the VFL value (VFL-TFL) are considered perennial ryegrass. This is consistent with the principle assumptions of the fluorescence test. For more information and supporting evidence see Appendix 1.

### Expected benefits of the proposed formula

- One of the benefits of the proposed formula is that it will replace the old formula, which was inadequate for the current types of rygrasses produced. Additionally, it was difficult to understand, learn or explain.
- The new formula follows strictly the basic assumptions of the fluorescence test, works for all samples and has the following advantages:
    - It provides more realistic estimates of annual and perennial types in the pure ryegrass seed component in the purity test.
    - The formula itself is simpler and easier to understand than the current formula, which is important for training and application by seed analysts.
    - It is user friendly because it orients the user to the specific formula and examples for annual or perennial ryegrass or mixture.
    - Its simplicity will contribute to better understanding and communication among seed producers, cleaners, laboratories, dealers and buyers.

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