

AOSA Rule Proposal

Purpose of Proposal: Clarification to section 5.2.b.2. of the rules and correcting “Example 8, Mixture of annual and perennial ryegrass”.

Present Rule:

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 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 = (%VFLA – %TFL)/100% × % pure ryegrass

% Perennial ryegrass = (100.00 % - 81.91%)/100% × 78.06% = 0.1809 × 78.06 % = 14.12%

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

Proposed Rule:

Examples with Blends and Mixtures of Annual and Perennial Ryegrass

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 weighted average of the** “expected” VFL values of various species (i.e., **mixture of** annual and perennial) or varieties (**blend** within the same species) may not predict the “actual” VFL of the seeds in the **bag container** 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** weighted average of 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 container**, 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.

Blends: When blending varieties within the same species (**all annual or all perennials**) with similar seed sizes and VFL values, using the **extrapolated weighted average of VFL for all varieties** may present a good approximation to the actual VFL of the blend in the **bag container**, and should be **calculated** (see example 6).

Mixtures: When mixing annual (big seeds with high VFL) with perennial (small seeds with low VFL) ryegrass, the **weighted average extrapolating an** “expected” VFL of all varieties in the mixture (based on percentage by weight) would be misleading. **This is** because of the large difference in seed sizes and VFL values which is calculated based on percentage by number. In such cases, **do not calculate the weighted average of the varieties in the mixture**. The best predictor of the fluorescence level of the seeds inside the **bag container** would be the TFL of the mixed product (see example 7, 8). If this is in doubt, simply conduct a supplementary **TFL** test for more precise determinations.

In summary, in case of blend samples, weighted average of VFL of all varieties in the blend should be calculated, but in case of mixture of annual and perennial varieties, weighted average should not be calculated since the TFL is the most reliable indicator to determine whether the mixture behaves like annual or perennial.

Example 8. Mixture of annual and perennial ryegrass in the following proportions (sec. 5.2.b.2).

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~~ 77.75%

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 ~~weighted average of VFL for all the varieties in~~ the mixture would be misleading, ~~so do not calculate it.~~

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 = $[(\%VFL_A - \%TFL)/100\%] \times \% \text{ pure ryegrass}$

% Perennial ryegrass = $[(100.00\% - 81.91\%)/100\%] \times \del{78.06\%} 77.75 = 0.1809 \times \del{78.06\%} 77.75 = \del{14.12\%} 14.06\%$

% Annual ryegrass = $\del{78.06\%} 77.75 - \del{14.12\%} 14.06 = \del{63.94\%} 63.69\%$

Table 14 I can be used to compare the % of PRG or ARG in the label (or the 1st test) to the 2nd test based on the percentage of the TFL.

Harmonization/Impact Statement:

ISTA does not have fluorescence test in their rules, therefore, this rule proposal is not relevant to ISTA. Federal Seed Act follows AOSA Rules in regard to fluorescence test calculations and they may want to consider the clarifications and corrections in this proposal. Canadian Methods and Procedures for Testing Seeds (M&P) use the fluorescence test in their rules to distinguish between annual and perennial ryegrass and may want to consider the clarifications and corrections in this proposal.

Supporting Evidence:

Recently, questions from seed analysts were sent to the statistics committee regarding the ambiguity of calculating the percentages of annual and perennial ryegrass in mixture samples in section 5.2.b.2 of the AOSA Rules, as well as the unclarity in Example 8. Therefore, this rule change proposal focused on adding clarification to the paragraphs that explain the calculations

pertaining to the mixture of annual and perennial samples in Examples 7 and 8.

The current rules indicated that “When mixing annual (big seeds with high VFL) with perennial (small seeds with low VFL) ryegrass, the extrapolating (or the weighted average) “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).

Therefore, we added in this proposal the following clarification statement: “In such cases, do not calculate the weighted average of the varieties in the mixture”. We also changed the word “extrapolated” to “calculating the weighted average” throughout all paragraphs as it is the correct terminology in this situation.

In regard to the blend samples (i.e., all varieties are annual, or all varieties are perennial) with similar seed sizes and VFL values, using the weighted average of VFL may present a good approximation to the actual VFL of the blend in the bag, and should be used (see example 6). We add in this proposal that: “the weighted average of all varieties included in the blend should be calculated”.

In addition, in this proposal we corrected the error in Example 8 in sec. 5.2.b.2. The Rule stated that the pure ryegrass component in the sample = 78.06%, the correct value is 77.75%. We corrected all calculations based on the new correct value.

In summary, we made it clear that in case of blend samples, weighted average of VFL of all varieties in the blend should be calculated, but in case of mixture of annual and perennial varieties, weighted average should not be calculated since the TFL is the most reliable indicator to determine whether the mixture behaves like annual or perennial. This is because of the difference in seed sizes among species, variability of VFL within each variety, and the sampling variation.

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