

2016 Proposal 17 Supporting Evidence

Appendix 17

Change the AOSA Formula used for the “Application of Fluorescence Test Results to Sample Purity”

Objective

The objective of this proposal is to replace the current formula in section 5.2.b (2) of the AOSA Rules Vol.1 that addresses the “Application of Fluorescence Test Results to Sample Purity”.

Rationale

Annual ryegrass (ARG) cannot be differentiated from perennial ryegrass (PRG) by seed morphology. The fluorescence test is used to distinguish between the two types based on the assumption that the roots of the annual type fluoresce close to 100% and close to 0% in the perennial type upon exposure to UV light. The current AOSA Rules Vol.1, sec 5.2.b (2) requires the use of the following formulae to determine the proportions of annual and perennial types in the pure ryegrass seed component in the purity test.

The percentage of perennial ryegrass is calculated as follows:

$$\% \text{ perennial ryegrass} = \frac{\% \text{ VFl (annual)} - \% \text{ TFl}}{\% \text{ VFl (annual)} - \% \text{ VFl (perennial)}} \times \% \text{ pure ryegrass}$$

Where VFl is varietal fluorescence and TFl is test fluorescence.

$$\% \text{ annual ryegrass} = \% \text{ pure ryegrass} - \% \text{ perennial ryegrass}$$

This formula works well when the Varietal Fluorescence Level (VFL) value of PRG varieties is zero. However, in recent years, new PRG varieties with higher VFL have been developed (currently there are PRG varieties with 10, 20, 30, 50, and even 80% VFL). It has been discovered that, as the VFL values gradually increase, the formula provides distorted results. The unreliable results create problems to both seed producers and buyers and affect the “truth in labeling” principle. Thus, the formula needs to be corrected.

The application of fluorescence test in purity testing uses the following assumptions:

- a) Most PRG varieties do not have the root fluorescence trait. If the VFL of a PRG variety is not described, the AOSA Rules assumes a “0” VFL value. Whenever the VFL is “0”, any fluorescing seedling found in a PRG sample is considered as annual type.

- b) Some perennial varieties have the root fluorescence trait. The percentage of the fluorescent seedlings considered perennial in each variety is described by the breeder of the variety and expressed as the “VFL” value. In such varieties, the test fluorescence (TFL) result (i.e., the number of seedlings with fluorescent roots at the end of germination test period) of a sample is compared to the described VFL value of the variety to determine the proportions of annual and perennial types in the sample. For example, when a variety is described to have 5% VFL, it means that up to 5% of seedlings with fluorescing roots are considered perennial type.
- c) If the TFL value is equal to or lower than the VFL value, then all fluorescing seedlings are considered perennial type. In this case, there is no need to apply the formula. However, if the TFL result is higher than the VFL, the excess seedlings with fluorescent roots ($\%TFL - \%VFL$) are considered annual type and a formula has to be applied.

Example 1. A variety has a 100% pure ryegrass, VFL is 10%, and a sample of that variety had TFL result of 9%. What is the proportion of annual and perennial types in the sample?

- This variety is described to have 10% VFL, which means that up to 10% of seedlings with fluorescing roots are considered perennial type. Since the TFL value (9%) is less than the VFL value (10%), then all fluorescing seedlings are considered perennial type.
- Obviously, the rest of the seedlings, which did not fluoresce (91%), are considered perennial.
- Therefore, all normal seedlings (9% fluorescence + 91% non-fluorescence) are considered perennial type and thus all pure ryegrass seed is considered PRG.

Example 2. A variety has a 100% pure ryegrass, VFL is 10% and a sample of that variety had TFL result of 20%. What is the proportion of annual and perennial types in the sample?

- In this case, seedlings with fluorescing roots up to 10% are considered perennial ryegrass.
- Seedlings with fluorescing roots in excess of VFL, i.e., $20\% - 10\% = 10\%$, are considered annual ryegrass.
- Obviously, seedlings with non-fluorescing roots, i.e., 80%, are considered perennial ryegrass.
- The total PRG seedlings in this sample would be = 90%. This is because 10% of the seedlings with fluorescing roots are considered perennial type based on the VFL description of that variety plus the 80% of seedlings with non-fluorescing roots. A schematic diagram of this example is presented in Figure 1.

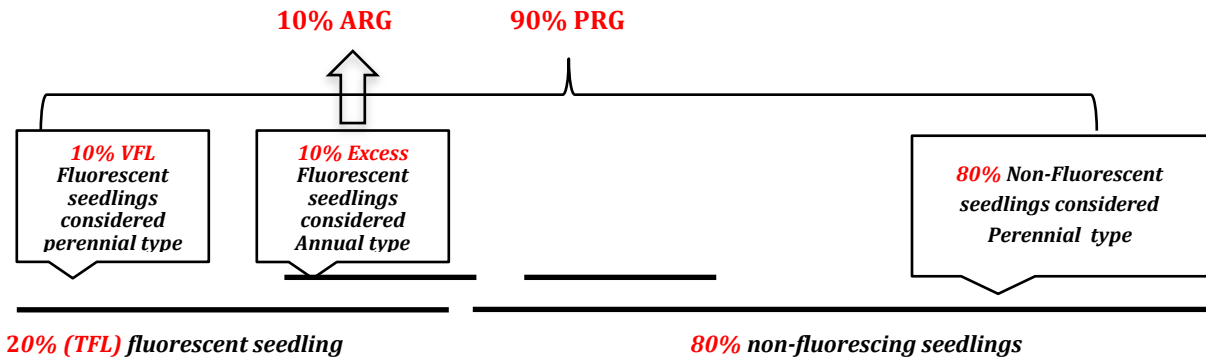


Figure 1. Schematic diagram demonstrating the percentage of ARG and PRG seedlings in a ryegrass sample that has VFL value of 10% and TFL value of 20%, and pure ryegrass of 100%.

Based on the schematic diagram and example 2, the following proposed formula is used to determine the proportions of annual and perennial types in the pure ryegrass seed component:

For perennial ryegrass varieties, the proposed formula is:

$$\% \text{ Annual ryegrass} = (\% \text{ TFL} - \% \text{ VFL}) / 100\% \times \% \text{ Pure ryegrass}$$

$$\% \text{ Perennial ryegrass} = \% \text{ Pure ryegrass} - \% \text{ Annual ryegrass}$$

Application of this formula to Example 2:

$$\% \text{ Annual ryegrass} = (\% \text{ TFL} - \% \text{ VFL}) / 100\% \times \% \text{ pure ryegrass}$$

$$\% \text{ Annual ryegrass} = (20\% - 10\%) / 100\% \times 100\% = 0.10 \times 100\% = 10\%$$

$$\% \text{ Perennial ryegrass} = \% \text{ pure ryegrass} - \% \text{ Annual ryegrass} = 100\% - 10\% = 90\%$$

However, if we apply the **current AOSA formula** to Example 2, the following results will be obtained:

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

$$\% \text{ Perennial Ryegrass} = \frac{100 - 20}{100 - 10} \times 100\% = 0.8889 \times 100\% = 88.89\%$$

In this formula, the numerator (100 - 20) is incorrect because the perennial ryegrass should be 10% plus 80% (see example 2 and Fig.1). Likewise, the denominator (100-10) is incorrect because it should be 100%, which represents all seedlings fluorescing and non-fluorescing.

The estimation of annual ryegrass would be:

$$\% \text{ Annual ryegrass} = 100\% - 88.89\% = 11.11\%$$

This estimate is distorted because we are subtracting a distorted % PRG value from 100%. Thus, both the estimation of PRG (88.89%) and ARG (11.11%) are distorted.

Effect of increasing VFL levels on the distortion of results when the current AOSA formula is used

Table 1 presents a theoretical demonstration of the effect of increasing VFL levels on the distortion of purity results, if the current AOSA formula is used. In column 1, the table shows the VFL values of four PRG varieties at 0, 10, 50 and 80%. In column 2, the example assumes that the TFL result in every case is 10% more than the respective VFL value of each variety. The pure ryegrass component in each sample is assumed to be 100% for simplicity of calculation. Columns 5 and 8 present the estimated annual and perennial types in each sample using the current AOSA formula. Columns 4 and 7 present the estimated annual and perennial types in each sample using the proposed formula. Columns 6 and 9 demonstrate the magnitude of distortion represented by the difference in estimates between the current AOSA formula and the proposed formula.

The results show the following:

- When the VFL value is “0”, both, the current AOSA formula and the proposed formula produce the same results.
- The proposed formula estimates the PRG as 90% and the ARG as 10% correctly in every case (columns 4 and 7) at any VFL value. However, when the current AOSA formula is applied, there is a gradual distortion of the results when the VFL values are 10% or above (columns 5 and 8).

Table 1. Comparison of the current AOSA formula and the proposed formula on the estimated annual and perennial ryegrass results. Theoretical numbers.

(1) VFL	(2) TFL	(3) TFL - VFL	(4) Estimated PRG with Proposed formula	(5) Estimated PRG with current AOSA Formula	(6) Under- estimation of PRG with AOSA Formula	(7) Estimated ARG with Proposed Formula	(8) Estimated ARG with current AOSA Formula	(9) Over- estimation of ARG with AOSA Formula
%								
0	10	10	90	90	0	10	10	0
10	20	10	90	88.89	1.11	10	11.11	1.11
50	60	10	90	80	10	10	20	10
80	90	10	90	50	40	10	50	40

- When the AOSA formula is applied, the perennial type is underestimated and the annual type is overestimated in the ryegrass samples (columns 6 and 9). The magnitude of underestimation is detectable at VFL of 10% and becomes dramatic above 50% VFL

(column 6). The overestimation of annual contaminant follows the same pattern (column 9). These results demonstrate that the current AOSA formula was not intended for perennial ryegrass varieties with high VFL.

Table 2 presents actual examples for the effect of increasing VFL levels on the distortion of purity results, when the current AOSA formula is used. The table used real test results of 10 samples tested at the OSU seed lab representing 5 different varieties. The PRG varieties used for the comparison of results between the current AOSA and the proposed formulae have VFL values of 0%, 5.88%, 10.03%, 48.69% and 81.78%. Two TFL results, one low and one high, were used within each of the five varieties. Samples with 98% or higher pure ryegrass component were used in these comparisons. For simplicity in presentation, only the results of the estimated ARG contents are presented.

Table 2. Estimated annual ryegrass contents in 10 perennial ryegrass samples representing 5 varieties with different VFL values and TFL results.

(1) VFL of 5 known varieties	(2) TFL of 2 samples of each variety (low & high TFL)	(3) TFL - VFL	(4) Estimation of ARG with proposed formula	(5) Estimation of ARG with current AOSA Formula	(6) Over- estimation of ARG using AOSA Formula
%					
0	1.38	1.38	1.36	1.36	0
	16.00	16	15.83	15.83	0
5.88	7.76	1.88	1.85	1.96	0.11
	22.98	17.1	16.66	17.7	1.04
10.03	11.83	1.8	1.79	1.99	0.2
	18.11	8.08	7.96	8.84	1.05
48.69	52.77	4.08	4.08	7.94	3.86
	85.00	36.31	36.26	70.67	34.41
81.78	84.41	2.59	2.59	14.21	11.62
	95.89	14.11	14.10	77.36	63.27

The results demonstrate the following:

- When the VFL value is “0”, both, the current AOSA formula and the proposed formula produce the same results. This is consistent with the findings in Table 1.
- When the VFL value is larger than “0”, the estimated annual ryegrass based on the proposed formula is correct because only those TFL values exceeding the VFL values are assumed to be annual (column 4). This estimation is correct in all cases, regardless of the VFL level. Noticeably, the estimated amount of the annual type varies proportionally with the level of TFL divergence from the VFL of each variety.

- The AOSA formula, however overestimated the annual type in every variety that has VFL values above “0” (column 5). The magnitude of overestimation increases significantly as the VFL values increase (column 6).

The results presented in Fig.1 and Tables 1 and 2 demonstrate that the current AOSA formula works well in PRG varieties with VFL values around “0”. However, it distorts the estimated proportions of annual and perennial ryegrass in a sample when the variety has VFL values above “0”. The distortion is smaller at lower VFL values and increases gradually at higher VFL values.

Based on all the above information, the proposed formulae that should be used to determine the proportions of annual and perennial types in the pure ryegrass seed component are:

For *perennial ryegrass varieties*, the formula is:

$$\% \text{ Annual ryegrass} = (\% \text{ TFL} - \% \text{ VFL}_P) / 100\% \times \% \text{ Pure ryegrass}$$

$$\% \text{ Perennial ryegrass} = \% \text{ Pure ryegrass} - \% \text{ Annual ryegrass}$$

For *Annual ryegrass varieties*, the formula is:

$$\% \text{ Perennial ryegrass} = (\% \text{ VFL}_A - \% \text{ TFL}) / 100\% \times \% \text{ Pure ryegrass}$$

$$\% \text{ Annual ryegrass} = \% \text{ Pure ryegrass} - \% \text{ Perennial ryegrass}$$

Where, *TFL* is the test fluorescence value, *VFL_P* is the perennial ryegrass varietal florescence value and *VFL_A* is the annual ryegrass varietal fluorescence value.

Expected benefits of the proposed formulae

- The proposed formulae would replace the current AOSA formulae that became inadequate for PRG varieties with high VFL descriptions.
- The new formulae follow the basic assumptions of the fluorescence test and work for all ryegrass varieties with low and high VFL values. In addition, the proposed formulae have the following advantages:
 - Provide more realistic estimates of annual and perennial types in the pure ryegrass seed component in the purity analysis.
 - Simple and easy to understand compared to the current formulae. This is important for training and application by seed analysts.
 - User friendly as shown in the examples included in the proposed rule change for annual, perennial or mixture ryegrass samples.

- Contribute to better understanding and communication among seed producers, cleaners, laboratories, dealers and buyers.