Ryegrass Annuality & Perenniality Testing
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Variety Fluorescence Testing

- Seeds from a minimum of three lots, from at least two generations (one of which must be Breeder), are fluoresced, then grown out in the greenhouse.
- This sets the VFL* (Variety Fluorescence Level), which is used to calculate purity results with fluorescence tests using a formula from the AOSA Rules.
- Visit www.aosca.org for more information and biannual deadlines. Samples must be submitted to the lab at least 2 months before deadline.

Fluorescence

- AOSA Rules assume that beyond the VFL*, any annual seedlings will fluoresce, and perennial seedlings will not. If no VFL is established (or sample submitted as VNS), the rule assumes all fluorescing seedlings are annual.
- 400 Seeds are planted on white filter paper, germinated, and then the seedlings’ roots are evaluated under black light.
- There is a formula that uses the mechanical purity %, the VFL %, and the TFL* % to calculate the percentages of annual and perennial ryegrass in a sample.

Fluorescence Grow-out

- Standard AOSA grow-out: the suspect seedlings are planted in the greenhouse & grown out for approx. 42 days.
- The results are used to revise the mechanical purity, and a new report is issued.

400-Seed Grow-out

- 400-Seed or “direct” grow-out (used for very high-fluorescing perennials, or customer request): 400 seeds are directly planted in organic media in the greenhouse, and evaluated in approx. 42 days.

RAD: Ryegrass Allelic Discrimination

- From a 400-seed fluorescence test, the suspect seedlings, as well as control seedlings, are sent for an allelic discrimination (DNA) test.
- The numbers of annual-like, perennial-like, and hybrid alleles are determined.
- These numbers are used to revise the mechanical purity, and a new report is issued.

*Acronyms:
VFL = Variety Fluorescence Level (the inherent fluorescence of a variety, as published by AOSCA)
TFL = Test Fluorescence Level (the actual % fluorescence found in a 400 seed fluorescence test)
**Scenario 1**
Purity & fluorescence

- Lab conducts purity test, and reports % of ryegrass with “mechanical” statement
- Lab conducts germination & fluorescence tests & discards seedlings at end of test
- Lab calculates purity and fluorescence using formula from AOSA Rules, and sends report out
- If customer needs a grow-out or RAD, a new fluorescence must be conducted

**Benefits**
- Cheapest & fastest option
- Sufficient when TFL is less than VFL, or TFL falls within Certification tolerance

**Scenario 2**
Purity, fluor., & fluor. grow-out or RAD

- Lab conducts purity test, and reports % of ryegrass with “mechanical” statement
- Lab conducts germination & fluorescence tests, and sends out report
- Lab conducts grow-out or RAD regardless of % contaminants
- Lab sends out new report, with revised percentages

**Benefits**
- More streamlined, test conducted regardless of fluorescence level
- More accurate than with FL alone
- Grow-out will incur hourly charges if fluorescence exceeds 15%
- RAD results ready within a couple of days; price depends on # of seedlings

**Scenario 3**
Purity, fluor., & sequential fluor. grow-out or RAD

- Lab conducts purity test, and reports % of ryegrass with “mechanical” statement
- Lab conducts germination & fluorescence tests, and sends out report
- Unless otherwise requested, lab proceeds with grow-out or RAD if contaminant exceeds certification standards (Certified generation)* (Lab will not call)
- Lab sends out new report, with revised percentages.

**Benefits**
- More accurate than with FL alone
- Call us for evaluation if Foundation or Registered generation
- Grow-out will incur hourly charges if fluorescence exceeds 15%
- RAD results ready within a couple of days; price depends on # of seedlings

**Scenario 4**
High fluorescing perennials

- 400-seed grow-out

- Lab conducts fluorescence test, but does not use it to calculate the purity
- Simultaneously, the lab plants 400 seeds directly in the greenhouse
- Plants are evaluated in approx. 42 days
- Results are used to calculate purity percentages, and a revised report is issued

**Benefits**
- Most accurate test because of the large sample size
- Saves time
- Will not incur hourly charges
- This approach can be used for any sample when customer needs higher levels of accuracy & confidence

*Maximum other ryegrass allowed in Certified generation (Blue Tags):
3% Annual contaminant in Perennial lot.
2% Perennial contaminant in Annual lot.